PB-238 487

RESEARCH AND DEVELOPMENT OF A SELECTIVE ALGAECIDE TO CONTROL NUISANCE ALGAL GROWTH

B. L. Prows, et al

Pacific Northwest Environmental Research
Laboratory

Prepared for:

Environmental Protection Agency
Office of Water Research and Technology
Dow Chemical Company

August 1974

DISTRIBUTED BY:

NTIS
National Technical Information Service
U. S. DEPARTMENT OF COMMERCE

SELECTED WATER RESOURCES ABSTRACTS
INPUT TRANSACTION FORM

1. Report No.

2.

PB 238 487

W 75-03046

4. Title
RESEARCH AND DEVELOPMENT OF A SELECTIVE ALGAECIDE TO CONTROL NUISANCE ALGAL GROWTH,

5. Report Date

6.

8. Performing Organization Report No.

7. Author(s)
Prows, B. L. and W. F. McIlhenny

9.
The Dow Chemical Company,
~~Texas Division~~
Freeport, Texas. ~~77541~~

10. Project No. Program Element 1BA031

11. Contract/Grant No.
EPA
Contract No. 68-01-0782

12. ~~USEPA, Office of Research and Development~~

13. Type of Report and Period Covered

15. Supplementary Notes
Environmental Protection Agency, Ecological Research Series, report ~~number~~
EPA 660/3-74-019, August 1974. 221p, 14fig, 44tab, 44ref, 5 append.

16. Abstract
The primary objective of this project was to determine under natural, open-field conditions, the efficacy of two candidate algaecides, Compound No. 23 (2,5-Dichloro-3,4-dinitrothiophene) and No. 73 (p-Chlorophenyl-2-thienyl iodonium chloride) from Phase II of the multiple phase developmental program. Specific efforts were also directed toward further delineation of the toxicological and environmental persistence properties of the candidate compounds, as well as further development of a possible biological-chemical control system.

Data from the field tests conducted under a wide variety of conditions in four geographically diverse regions of the United States revealed a distinctive pattern of selective blue-green algal control for both experimental compounds. Compound No. 23 was eliminated from the test series due to unacceptable fish toxicity.

A whole-pond field study involving the use of a phagocytic organism, Ochromonas ovalis, as a biological control system, was inconclusive due to the apparent inability of the organism to survive under the existing environmental conditions.

Continued laboratory screening tests of some 70 additional compounds produced two additional candidate compounds, No. 136 (2,2'-(1,2-Ethenediyl)bisbenzoxazole) and No. 176 (1,2-Dichloro-4-(isothiocyanatomethoxy) benzene).

Continuation of testing of candidate compounds under field conditions is recommended. (EPA) —

17a. Descriptors
*algicides, *nuisance algae, *biocontrol, *toxicity, environmental ~~persistence~~ control, Organic compounds, Cyanophyta, Laboratory tests, Water pollution control, *On-site tests, Water pollution effects, North Carolina, Minnesota, Oregon, Chemcontrol

17b. Identifiers
Chowan River (NC), Lake Sallie (MN), Diamond Lake (OR), *Algal control
Algal growth, Phagocytic organisms, Ochromonas ovalis

17c. COWRR Field & Group 05C, 04A, 05G

Copy Available from GPO Sup. Doc. as EP1.23:660/3-74-019, $2.95
microfiche from NTIS as PB

19. Security Class. (Report)

20. Security Class. (Page)

21. No. of Pages 233

22. Price MF: $2.25

Send To:
WATER RESOURCES SCIENTIFIC INFORMATION CENTER
U.S. DEPARTMENT OF THE INTERIOR
WASHINGTON, D.C. 20240

Abstractor Bernard L. Prows

Institution The Dow Chemical Company

Reproduced by
NATIONAL TECHNICAL INFORMATION SERVICE
US Department of Commerce
Springfield, VA. 22151

EPA-660/3-74-019
August 1974

RESEARCH AND DEVELOPMENT OF A SELECTIVE ALGAECIDE TO CONTROL NUISANCE ALGAL GROWTH

BY

B. L. PROWS

W. F. McILHENNY

CONTRACT NO. 68-01-0782
PROGRAM ELEMENT 1BA031
ROAP/TASK 21 AIZ 06

PROJECT OFFICER
THOMAS E. MALONEY
PACIFIC NORTHWEST WATER LABORATORY
WATER QUALITY OFFICE
ENVIRONMENTAL PROTECTION AGENCY
CORVALLIS, OREGON 97330

PREPARED FOR

OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C. 20402 - Price :

1a

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Monitoring, Environmental Protection Agency, have been grouped into five series. These five broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The five series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial and atmospheric environments.

EPA REVIEW NOTICE

This report has been reviewed by the Office of Research and Development, EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

ABSTRACT

The primary objective of this project was to determine under natural, open-field conditions, the efficacy of two candidate algaecides, Compound No. 23 (2,5-Dichloro-3,4-dinitrothiophene) and No. 73 (p-Chlorophenyl-2-thienyl iodonium chloride) from Phase II of the multiple phase developmental program. Specific efforts were also directed toward further delineation of the toxicological and environmental persistence properties of the candidate compounds, as well as further development of a possible biological-chemical control system.

Data from the field tests conducted under a wide variety of conditions in four geographically diverse regions of the United States revealed a distinctive pattern of selective blue-green algal control for both experimental compounds. Compound No. 23 was eliminated from the test series due to unacceptable fish toxicity.

A whole-pond field study involving the use of a phagocytic organism, Ochromonas ovalis, as a biological control system, was inconclusive due to the apparent inability of the organism to survive under the existing environmental conditions.

Continued laboratory screening tests of some 70 additional compounds produced two additional candidate compounds, No. 136 (2,2'-(1,2-Ethenediyl)bisbenzoxazole) and No. 176 (1,2-Dichloro-4-(isothiocyanatomethoxy) benzene).

Continuation of testing of candidate compounds under field conditions is recommended.

TABLE OF CONTENTS

Page

Abstract ii

List of Figures iv

List of Tables v

Acknowledgements ix

Sections

I Conclusions 1

II Recommendations 3

III Introduction 4

IV Experimental Procedure 15

V Discussions 29

VI References 121

VII Appendices 125

LIST OF FIGURES

Figure Number		Page
1	Open Field Test System	22
2	North Carolina Test Site	31
3	Chowan River - First Test, Test Compound No. 23 vs. *M. Cyanea* and *M. incerta*	35
4	Chowan River - Second Test, Test Compound No. 73 vs. *M. cyanea* and *M. incerta*	38
5	Compound Depletion Patterns, Chowan River - First Test	41
6	Minnesota Test Site.	49
7	Lake Sallie and Muskrat Lake - Linological Sampling Stations and Algaecide Testing Stations.	50
8	Compound Depletion Patterns, Second Minnesota Test - Muskrat Lake, Test Compound No. 73. . . .	60
9	Oregon Test Site	66
10	Diamond Lake	67
11	Northern Texas Test Site	81
12	Whole Pond Test - North Texas, Compound No. 73 vs. *Microcystis*.	87
13	Fish Toxicity Tests - Blue Gills, Test Compound No. 73	95
14	Influence of Three Test Compounds on the Phagocytic Activity of *Ochromonas danica* at 0.2 ppm .	112

LIST OF TABLES

Table Number		Page
1	Chowan River - First Test, *Anabaena spp.* and *Microcystis montana*	33
2	Chowan River - Second Test, *Oscillatoria tenuis* and *Agmenellum quadruplicatum*	34
3	Chowan River - First Test, *Microcystis cyanea* and *Microcystis incerta*	36
4	Chowan River - Second Test, *Anabaena spp.* and *Microcystis cyanea*	39
5	Chowan River - Second Test, *Oscillatoria planctonica* and *Microcystis incerta*	40
6	Chowan River - Second Test, Compound Depletion Patterns	42
7	Chowan River - First Test, Compound Depletion Patterns	43
8	Degradation-Absorption Check on Compound No. 73 Under Controlled Conditions	45
9	Chowan River - Second Test, Dissolved Oxygen Levels	47
10	Lake Sallie - First Test, Compound Depletion Patterns	52
11	Lake Sallie - First Test, Test Compound Action on Blue-Green Algae *Aphanizomenon* and *C. naegelianum*	53
12	Lake Sallie - First Test, Test Compound Action on Blue-Green Algae *Anabaena* and *Microcystis*	54
13	Lake Sallie - First Test, Total Blue-Green Algae	56
14	Lake Sallie - First Test, Total Algal Cell Counts x 10^2/ml	57

LIST OF TABLES continued

Table Number		Page
15	Lake Sallie - First Test, Test Compound Action on *Oocystis* and *Fragillaria*	58
16	Muskrat Lake - Second Minnesota Test, Compound Depletion Patterns.	61
17	Muskrat Lake - Second Minnesota Test, *Anabaena* and *Aphanizomenon*	62
18	Muskrat Lake - Second Minnesota Test, *Coelosphaerium* and *Raphidiopsis*.	63
19	Muskrat Lake - Second Minnesota Test, *Oscillatoria*	64
20	Diamond Lake - First Test, Compound Depletion Patterns	69
21	Diamond Lake - First Test, Blue-Green Alga *Anabaena*	70
22	Diamond Lake - First Test, Diatoms - *Synedra* and *Stephanodiscus*	71
23	Diamond Lake - First Test, Fluorometric Relative Intensity Readings.	72
24	Diamond Lake - First Test, Chlorophyl "A" and Carotenoid Levels.	74
25	Diamond Lake - Second Test, Compound Concentrations	76
26	Diamond Lake - Second Test, Blue-Green Alga *Anabaena*	78
27	Diamond Lake - Second Test, Green Alga *Gleocystis*	79
28	Diamond Lake - Second Test, *Staurastrum* and *Stephanodiscus*	80
29	North Test Test Site - Monitored Test Compound Levels	84

LIST OF TABLES continued

Table Number — Page

30 North Texas - Whole Pond Test, *Oscillatoria* and *Agmenellum* 85

31 North Texas - Whole Pond Test, *Anabaena* and *Microcystis*. 88

32 Phase III Field Test Results, Algal Control - Compound No. 73. 89

33 Phase III Field Test Results, Algal Control - Compound No. 23. 90

34 Fish Toxicity Tests - Blue Gills, Compound No. 23 92

35 Fish Toxicity Tests - Blue Gills, Compound No. 73 94

36 Fish Toxicity Tests - Rainbow Trout, Compound No. 73 96

37 Laboratory Screening Tests, Test Compounds versus *Anabaena flos-aquae* 97

38 Laboratory Screening Tests, Test Compounds versus *Microcystis aeruginosa*. 104

39 Algaecidal Activity of $CuSO_4 \cdot 5H_2O$ Against Two Species of Blue-Green Algae. 106

40 Algaecidal Activity of Cutrine Against Two Species of Blue-Green Algae. 107

41 pH Sensitivity Tests on Compounds No. 23 and No. 73 Against *Microcystis* 108

42 Algaecidal Activity - Biomass Dependence Test versus *Anabaena flos-aquae* 109

43 Influence of Test Compound No. 117 at 0.2 ppm on the Phagocytic Activity of Four Species of *Ochromonas*. 111

LIST OF TABLES continued

Table Number | Page

44 Ochromonas Storage-Viability Study 114

ACKNOWLEDGEMENTS

The work done in connection with this project was coordinated by Dr. B. L. Prows, principal investigator, with the assistance of W. F. McIlhenny, project director. C. P. Ward assisted in the laboratory and field work.

We are indebted to each of the site investigators in the four principal test areas, for their interest, cooperation, and efforts in gathering background data and directing the field tests in their respective areas: Dr. B. J. Copeland and Jim McKenzie of the North Carolina State University; Dr. J. K. Neel and Dave Brakke of the University of North Dakota; personnel from the Lake Sallie, Minnesota State Fish Hatchery; Dr. H. Horton and Jim Rybock of Oregon State University; Dr. B. D. Vance and his graduate students from North Texas State University.

Appreciation is also expressed to the various state officials whose cooperation made the site utilization possible, and to Drs. M. J. Wynne and G. T. Cole of the Department of Botany, University of Texas at Austin, who served as consultants and investigators on Biological Algal Control mechanisms.

The support of The National Environmental Research Center of the United States Environmental Protection Agency, and of Dr. T. E. Maloney of the National Eutrophication program, who was the Federal Project Officer, is gratefully acknowledged.

SECTION I
CONCLUSIONS

Data collected under a wide variety of conditions in four geographically diverse regions of the United States indicate a rather distinctive pattern of blue-green algal control by both of the two prime candidate algaecidal compounds, No. 23, 2,5-Dichloro-3,4-dinitrothiophene; and No. 73, p-Chlorophenyl-2-thienyl iodonium chloride. Other supporting data regarding compound selectivity, environmental persistence and fish toxicity was definitive.

The following specific conclusions are reached:

1. Compound No. 73, having met all of the original objectives, has emerged as the best candidate. It is an effective chemical for algal control; it is safe to applicators, fish, and other higher aquatic plants and animals; it has a fairly rapid degradation pattern under open atmospheric conditions, with a half-life of one to two days; and it also exhibits a fairly high degree of specificity for the target algae, particularly Anabaena, Microcystis, Aphanizomenon, and Oscillatoria. It is relatively inactive against diatoms and most green algae.

2. Compound No. 23 also proved to be an effective algaecid and met most of the criteria, but was found to be toxic to fish life and thus had to be eliminated from the test program.

3. Through the continuing laboratory screening of 70 additional compounds; No. 136, 2,2'-(1,2-Ethenediyl)

bisbenzoxazole; and No. 176, 1,2-Dichloro-4-(isothiocyanatomethoxy) benzene; exhibited good algaecidal properties, showing 91 to 97 percent control of Anabaena at the 0.8 ppm level.

4. Continued efforts toward development of a biological-chemical control system have resulted in further investigations of the phagocytic algal organism, Ochromonas ovalis, which has proven to be a voracious feeder on the blue-green alga Microcystis aeruginosa, and have shown that Ochromonas can retain some viability for more than 70 days at room temperature when imbibed in activated charcoal or cotton and polyester fibers; whole pond field tests with this organism for control of Microcystis were unsuccessful as the Ochromonas was apparently unable to cope with the new environmental conditions present at the time.

SECTION II

RECOMMENDATIONS

In view of the positive results obtained thus far in the long-range development program, and the relatively good prognosis for successful achievement of the original objectives, it is recommended that the research program be continued at least to Phase IV, of the general development plan, under government funding. There are certain inherant uncertainties and areas which should be investigated in greater depth to determine whether the project should be advanced, terminated, or modified at the conclusion of Phase IV.

It is suggested that this next phase should include:

1. Additional field tests under natural conditions, especially in larger single ponds, small lakes, and fish hatchery empoundments. to obtain more information on the algal control efficacy of the candidate compound.

2. Further tests to determine the compound's environmental safety, including expanded animal and plant toxicities and compound degradation patterns under natural conditions.

3. Continued laboratory testing, as new compound structures shown to be potential algaecides are synthesized in proprietary programs, or otherwise become available for preliminary screening.

4. Continued investigation of the biological control phenomena by flagellated phagocytes, especially with regard to the development of practical application methods.

SECTION III

INTRODUCTION

All surface waters contain dissolved and suspended materials which serve as nutrients and help support the growth of algae and many other forms of aquatic life, the numbers and variety of which are determined by the amounts and kinds of nutrients which are available (Palmer, 1962). A certain amount of natural eutrophication in our fresh water systems is tolerable, and even desirable, for the support of fish life and the necessary accompanying biota. Excessive eutrophication, however, upsets the natural aquatic ecological balance and causes many troublesome aesthetic and economic problems.

In comparatively recent times the availability of adequate supplies of good quality fresh water has come to be regarded as one of our most valuable natural resources, and the control of nuisance algae growth and aquatic weeds is one of the major concerns of the Environmental Protection Agency (Prescott, 1970). To this end a cooperative plan of action has been formulated for the collection of a sufficient range of comparable data on the degree and extent to which nutrient loading in our fresh water lakes is correlatable with the rate at which eutrophication is developing (EPA, 1973).

Algae are found as common and natural inhabitants of all surface waters and are especially abundant where the water is exposed to direct sunlight. The portion of the available solar radiation spectrum utilized is in the visible, infrared, and ultraviolet regions, in the approximate ratio of 60:45:1, respectively (Brown, 1973).

To date more than 18,000 species of algae have been identified (Palmer, 1962) but only a relatively small number of these, principally the blue-greens, are considered to be notable nuisance species.

Unlike the other groups of small microscopic organisms all species of algae contain chlorophyll. Algae are responsible for an estimated 90 percent of all photosynthetic activity on the earth (Meyer, 1971). One pound of algae growth will produce about 15 pounds of oxygen (MacKenthun and Ingram, 1964).

The constant increase in urbanization, accompanied by changes in ground cover and surface soil, together with such phenomena as forest fires, over-grazing, deforestation, and agriculture, having increased run-off and reduced soil seepage to the extent that, as estimated by some authorities, the underground water table in the Eastern half of the U.S. has been lowered about 60 feet in the last 50 years (Palmer, 1962). This, in addition to a large increase in the use of ground waters, has created severe shortages in many areas. Thus, as population and industrial demands increase, attention has, of necessity, turned more and more to lakes, streams, and reservoirs in order to meet these needs.

A need for an efficient and economically feasible method for controlling certain algal species has arisen as a result of excessive eutrophication of natural waters due to the continued increase in the wastes produced by the human population as well as from indescriminate use of available water supplies.

Although there is no universal agreement among scientists and environmentalists as to the particular elements which are responsible for excessive algal populations, the most commonly suspended nutrients are phosphates and nitrates.

It has been observed that algae blooms are often associated with waters which receive sewage effluents or other waters which are rich in these components. Thus, it is widely believed that in many bodies of fresh water, growth of phytoplankton tends to be limited by the supply of inorganic phosphate (Talling, 1962). MacKenthun and McNab (1961) made studies of several Wisconsin stabilization ponds and concluded that the annual per capital contribution of soluble phosphorous and inorganic nitrogen was 1.1 and 4.1 pounds, resspectively.

When the physical and chemical conditions of a body of water become optimal for a particular algal species, proliferation may take place in such abundance as to produce visible aggregations of floating algal masses. To produce such "algal blooms" the combined growth conditions usually become optimal for only one species at a time. Thus an algal bloom usually contains one heavily predominant species.

Many types of algae, particularly the blue-greens, tend to impart obnoxious tastes and orders to the water, clog intake screens and rapid sand filters of water treatment plants, and produce unsightly collections of debris on shores, making the water unsuitable for many desired uses.

The presence of algae in water supplies has been known to cause digestive upsets or even death to certain warm-blooded animals due to toxic substances released to the water. A number of case histories are recorded in which certain genera of Cyanophyta (blue-green algae) such as Microcystis, Aphanizomenon and Anabaena are known to have caused animal deaths, particularly in areas where the wind may have concentrated the algae into leeshore areas (MacKenthun and Ingram, 1964).

A rather common occurrance resulting from heavy algal blooms is a severe fish kill due to the depletion of dissolved oxygen in the water. Even though algae do impart oxygen to the water, through photosynthesis during daylight hours, the metabolic and catabolic processes taking place in living plant cells continuously utilize oxygen both day and night. Should a heavy cloud cover reduce the oxygen-producing photosynthetic activity of the algae to an abnormally low level over a several-day period, the dissolved oxygen level during the night, when photosynthesis ceases and oxygen-consuming digestive processes still continue, may fall below the critical level required to sustain life in many species of fish, and massive fish kills may result. Game fish such as trout, which have high dissolved oxygen requirements are usually the first to be affected in such cases.

The pH of the water will tend to increase, as algae extract carbon dioxide from the water for photosynthetic action, thus reducing the amount of soluble carbonic acid in the water, as well as the intermediately soluble bicarbonates and the nearly insoluble monocarbonates, usually causing part of the latter to precipitate. Water storage and transport problems may also be caused by the depolarizing action of the oxygen produced during photosynthesis (Palmer, 1962).

The literature documents the damage to our inland waters in recent years as a result of excessive algal growth. For example, Lake Washington in 1959, contained a maximum phytoplankton population of 1.5 x 10^6 μ^3/ml, of which only 15 percent was made up of blue-green algae. By 1963, the phytoplankton population had increased ten-fold and consisted of 95 percent blue-greens (Bartsch, 1967).

In contrast to most species of non-flagellated algae which settle to the bottoms of lakes in calm weather, many planktonic blue-greens exhibit an "upside-down" characteristic, accumulating as dense scums on the surface, which may then be blown by the wind into thick windrows and piled upon the shorelines. Often an accompanying offensive "pig-pen" odor results from the decaying material (MacKenthun and Ingram, 1964). Also, bad tastes as well as decomposition products are imparted to the water which may become toxic to animals and humans alike (Bartsch, 1967).

The problems associated with nuisance algal control are being investigated through the National Eutrophication Research Program of the Environmental Protection Agency. Basically the investigative efforts fall into four broad categories: mechanical, biological, ecological and chemical. Mechanical approaches involve the engineering and design of machines and equipment for underwater mowing, raking, and harvesting of certain algae and higher aquatic weeds. Biological control mechanisms being investigated include viruses, insects, algae-eating fish, and phagocytic organisms. Ecological approaches include diversion of nutrient-rich waters, flushing of lakes and ponds with nutrient-poor water and nutrient-removal by aquatic plant crops, or by flocculation and adsorption methods. Due to the great variety and complexity of conditions in which algae problems may exist, it is unlikely that any one single approach to the control of unwanted algae would be suitable in all cases. Without question, one of the basic solutions to the control of algae lies in the control of nutrient levels in the water. The nutrients and the required nutrient levels are not well understood, and the difficulties involved in controlling excessive eutrophication are so great that alternative methods of algae control will likely persist in the future for a considerable length of time.

For many situations, a chemical approach to the control of nuisance algae growths will be suitable, particularly in smaller lakes, streams and impoundments where algae "blooms" are imminent and the need to restore ecological balance exists.

Chemical approaches (the use of algaecides) have not been as thoroughly investigated because of the difficulties encountered in developing a compound which will selectively kill or inhibit reproduction of the target algal species without adversely affecting the other more desirable forms of aquatic life.

For a number of years, the most widely used algaecidal compound on the market has been copper sulfate. Despite its extensive usage, copper sulfate has disadvantages such as toxicity to desirable aquatic life at higher concentrations, non-biodegradability, accumulations of copper salts in bottom muds, and corrosive properties to paint and equipment (Bartsch, 1954). Hasler (1947) and Kuentzel (1969) point out the possible deleterious effects of lake-bottom accumulations of copper sulfate on lake ecology. Investigations conducted in Wisconsin and Minnesota by Moyle (1949) indicate that certain algae, particularly the blue-green alga Aphanizomenon seem to have acquired an increased tolerance to copper sulfate as a result of many years of successive treatments; in this case up to five times as much copper sulfate was needed for control of Aphanizomenon as was required 20 years earlier.

There is no federally registered chemical available for the selective control of nuisance species of blue-green algae in surface waters which are to be used for potable purposes. A considerable amount of time and effort has been expended in recent years in search of a compound which would replace copper sulfate as an algaecide, and at the same time prove to be safe to non-target organisms, non-cumulative in the environment, and be economically feasible to use for such purposes (MacKenthun, et al, 1964).

It is important to understand that the development of a compound for widespread use in the environment, particularly where recreational and potable waters are concerned, is a long-range and expensive process.

Phase I of this contractual effort to "Develop a Selective Algaecide to Control Nuisance Algal Growth", was initiated April 5, 1969. The primary objective was to develop a compound which would control the growth of various species of blue-green algae effectively, safely, and economically, while exhibiting a minimum impact on other forms of life in the aquatic environment.

The following criteria were specified as general guidelines for compound selection and development. A satisfactory algaecidal compound must have:

1. High activity against the target algae, specifically, against selected species of blue-green algae.
2. Low toxicity levels for mammals, fish, and other desirable aquatic organisms.
3. Low toxicity to terrestrial plants
4. No questionable elements such as arsenic, mercury, or other heavy metals, in its structure.

The approach to the problem involved initially making a computer search of some 80,000 compounds in Dow's computer listing of compounds in order to select those having the highest probability of meeting the established criteria for an ideal algaecide. Most of these compounds had already been screened against at least one species of algae and also for activity against higher aquatic plants, fish, and some terrestrial plants and animals. A final hand selection was then made from the computer printout, eliminating those compounds which contained heavy metals and those which were likely to be costly, or which possessed inherent or demonstrated undesirable properties, such as high toxicities to fish, terrestrial plants, or mammals. The candidate compounds, thus selected, were then divided into several priority groups according to the established selection criteria.

Within the 12-month contractual period, 33 compounds were screened using the two target species of blue-green algae suggested by EPA's Federal Water Quality Office, Corvallis, Oregon. Each compound was tested against cultures of *Microcystis aeruginosa* and *Anabaena flos-aquae* at a concentration of 2.0 ppm in constant-temperature water bath shakers (24°C) at 80 oscillations per minute under a cool-white fluorescent light intensity of 100 foot-candles. Compound activity was expressed as percent control, as compared to control cultures which were run simultaneously under identical conditions. Cell growth was monitored by visual inspections, cell counts, and relative intensity readings using a fluoromicrophotometer. Those compounds which passed the first screening test with at least 80 percent control at 2.0 ppm against both test species were selected for more detailed testing at 1.0, 0.5, and 0.1 ppm. Of the 33 compounds tested, Compound Nos. 23 (2,5-Dichloro-3,4-dinitrothiophene), 15 ([5-Chloro-2-(p-nitrophenoxy)phenyl]phenyl iodonium chloride), 8 (4-Amino-2,5-dibromophenyl thiocyanate), and 24 (1,1-Dimethyl tetradecyamine hydrochloride) were effective at these concentrations and were selected as the prime candidate compounds for further research through Phase II of the long-range algaecidal development program (Prows, 1971).

A phagocytic organism, identified as Ochromonas ovalis, was discovered which showed promise as a means of controling Microcystis. In addition, it was found that the growth and activity of Ochromonas was enhanced by low concentrations of some of the test compounds being studied.

As specified in the contractual work statement efforts were to be directed toward the long-range goals of developing an algaecidal compound which would:

1. be safe to non-target organisms
2. exhibit a high degree of specificity for the target algae
3. be economical
4. be safe to applicators
5. be non-persistent in aquatic systems

Phase II of the algaecide development program was begun June 30, 1971. Initially a computerized structure search of more than 100,000 compounds was made in order to select the analogs of the following four Phase I prime candidates: 2,5-Dichloro-3,4-dinitrothiophene; [5-Chloro-2-(p-nitrophenoxy)phenyl] phenyl iodonium chloride; 4-Amino-2,5-dibromophenyl thiocyanate; and 1,1-Dimethyl tetradecylamine hydrochloride. This endeavor resulted in the selection of 1309 analogs, which were each subjected to rapid agar-plate screening tests. Forty-one compounds emerged from these tests as candidates for final laboratory screenings. Although six of these compounds showed high levels of activity against the target blue-green algal species, four proved to be unacceptable because of poor economic feasibility, environmentally unacceptable properties, or difficulty or danger in compound production.

At the conclusion of Phase II, Compounds No. 23 (2,5-Dichloro-3,4-dinitrothiophene) and 73 ([p-Chlorophenyl]-2-thienyl iodonium chloride) were selected as final candidates based on superior algaecidal activity, environmental acceptability, economic feasibility, and freedom from human health and handling hazards (Prows and McIlhenny, 1973).

Three additional species of Ochromonas were discovered during Phase II which exhibited phagocytic activity against Microcystis aeruginosa. However, the original species discovered during Phase I exhibited the greatest activity and showed some activity improvement when used with low levels of certain test compounds.

The Phase III effort was begun on November 28, 1972. The objectives of this third phase were to test the candidate compounds under natural field conditions to obtain information on the algal control efficiency, and of combined biological-chemical systems in naturally occurring field situations. The stability and environmental stability of the compounds of concern were to be determined.

The initial approach was to synthesize large enough quantities for use in field tests. Analytical test procedures were to be developed in order that the persistence, adsorption, and degradability rates could be followed during and after treatment. The compounds were then to be field tested in small lakes and ponds having blooms of nuisance algae. Careful monitoring as well as the analysis of water, bottom muds, and higher aquatic plants. Toxicity studies on fish and mammals were to be undertaken. The biological and chemical-biological systems using Ochromonas were also to be further studied.

SECTION IV

EXPERIMENTAL PROCEDURES

The primary objective of this research project was to determine under natural, open-field conditions, the efficacy of two prime candidate algaecides, No. 23 (2,5-Dichloro-3,4-dinitrothiophene) and No. 73 (p-Chloro-phenyl-2-thienyl-iodonium chloride) which were final candidates at the conclusion of the Phase II effort. Specific efforts were to be directed toward further delineation of the toxicological properties and environmental persistence of these compounds, with objectives focused on further defining an effective chemical or biological-chemical system which would control the growth of blue-green algae safely and economically while exhibiting a high degree of specificity for the target algae.

In order to have sufficient quantities of the candidate compounds to allow the toxicology tests to be run and to have sufficient for each of the four field tests planned for various sectors of the country, a re-synthesis of the two test compounds was necessary. Twenty pounds of Compound No. 23 was manufactured according to specifications by Pharm-Eco Corporation, Simi Valley, California. A like quantity of Compound No. 73 was made in several successive batches at Dow's Organic Synthesis Labs in Midland, Michigan.

ANALYTICAL PROCEDURES

During the developmental stages of a pesticide, particularly if it is to be used in the aquatic environment, it is mandatory that a fast and accurate means be developed for detecting compound residuals as a function of elapsed time.

In this case the procedure had to be capable of monitoring traces of the compound in very dilute concentrations, preferably in the parts per billion range, since initial treatment concentrations were planned at around 1.0 ppm, and it was desirable to follow the compounds' persistence, adsorption, and degradation rates for several weeks following each treatment.

COMPOUND NO. 23

Both test compounds were examined by differential pulse polarography but the lowest detection limit achievable for Compound No. 73 was 3.0 ppm and the method was therefore unacceptable for this compound. The instrument used was a Princeton Applied Research Model 174 Polarographic Analyzer equipped with Model 174/70 drop timer to record the differential pulse polarograms.

A standard solution of Compound No. 23, prepared in methanol, was found to possess two polarographic waves in a sodium acetate supporting electrolyte, with half-wave potentials of -0.13 and 0.27v versus a saturated calomel reference electrode (SCE). Sodium acetate itself gave no response in this potential region, but when high instrument sensitivities were employed to achieve the desired response, interference due to impurities appeared. After electrochemical purification for a week (or longer), the sodium acetate was found to be free of interferences at the desired level of sensitivity. When laboratory distilled water and purified sodium acetate were used, a useful response was obtained from 20 ppb of the compound. Instrument response was linear with concentrations from 20 ppb to 20 ppm.

A sample of Tittabawassee River water was obtained upstream from Dow in Midland, Michigan, to check for interferences from natural waters. Purified sodium acetate was added to the river water and a polarographic scan was recorded. No interferences were observed in the potential region of interest.

At this point, it was desired to establish the stability of solutions of the compound so that proper shipping arrangements could be made, since test sites were scheduled for various parts of the United States. Initial dosage levels were expected to be 1.6 and 0.8 ppm, and therefore, a 0.6 ppm stock solution was selected for stability studies. Aliquots of this solution were determined polarographically at various time intervals. The compound remained stable at this concentration for at least a week under ordinary laboratory conditions.

Stability was also checked in Tittabawassee River water and similar results were obtained, although the background signal from untreated water increased with time, which would limit detection of trace amounts. Stability was also checked on refrigerated and frozen samples since it was planned to ship samples packed in dry ice. No adverse effects were observed.

From this procedure it was concluded that 2,5-Dichloro-3,4-dinitrothiophene could be determined polarographically in natural waters down to the 20 ppb level. The method proved to be quite specific, due to the presence of two polarographic waves.

COMPOUND NO. 73

Liquid chromatography was finally selected as the analytical method of choice for determination of Compound No. 73. The test compound concentrations were monitored at the elution end of the column by a Perkin Elmer "1250" ultraviolet detector at the 254 nanometer range.

The original gradient elution system developed was later modified by a more efficient and time-saving procedure. In the gradient method, the water sample was injected into a strong cation exchange resin (VYDAC-SCX). The water and organics were first eluted with a 1:1 water/methanol solution; the iodonium chloride, which had been retained on the column was then gradient eluted with a dilute (0.02 M) sodium perchlorate solution in 1:1 methanol/water. It was also found that methanol was as effective a mobile phase solvent as the water/methanol system. After the iodonium chloride was eluted, the column was then washed for ten minutes with the original solvent to remove all sodium ions. Due to the column washings, associated with the gradient system, a sample run lasted 20 to 30 minutes. Thus only about 15 samples, plus the appropriate standards, could be run per day with this method.

Recent work with chlorophenols has demonstrated that pentachlorophenol and tetrachlorophenol can be separated by ion exchange chromatography using constant composition elution with a weakly ionic mobile phase. Applying this principle to the iodonium chloride/water system, it was found that by using a constant composition mobile phase consisting of 0.01 M sodium perchlorate in methanol, the iodonium chloride was sufficiently retarded in passing through the column to be separated from the unretarded water and organics. With this method a

30-sample shipment could be analyzed in three hours. There were also no diffuse negative peaks associated with the solvent change preceding the iodonium chloride peak. The ionic strength needed to just separate the bacteriostat from the water/organics was constant for a given column but changed when a new column was packed.

A series of standard solutions was prepared and chromatographed with this technique. The chromatographic response was found to be linear in the 0.3 to 5.0 ppm range. For a given standard the reponse was also found to be reproducible to within ±1.5 percent of the mean response.

Thus, the analysis of 4-Chlorophenyl-2-thienyl iodonium chloride in natural water was simplified by replacing a two-solution gradient elution system with a single-solution procedure. The resulting 80 percent reduction in analysis time permitted prompt analysis of large numbers of samples, thereby circumventing problems of degradation in storage. The linearity and reproducibility of this method was found to be very good with standard solutions.

FIELD TESTS

Selection of Sites

It was proposed that test sites be selected in at least four different sectors of the United States so as to provide a good climatic cross-section with naturally occurring algal blooms. The general areas were selected with the advise of EPA personnel.

Specific sites were selected in connection with algologists whose previous work and interest had been associated with the limnological aspects of lakes and streams in the local areas where the particular Universities were located. The following criteria were used as a basis for selection of the test sites:

1. past history of algal populations and blooms
2. freedom from animal and human interactions
3. proximity of test sites to research personnel and laboratory facilities
4. prognosis of appropriate agency approval for permission to perform the tests

On a site-selection tour of the proposed test areas early in May, 1973, final details and arrangements for carrying out the field testing programs were made with the appropriate university investigators. Suitable bodies of water with reasonable proximities to the institutions concerned were chosen.

The following is a list of the selected sites, together with the university professors cooperating as area investigators:

1. Chowan River, North Carolina
   Dr. B. J. Copeland, University of North Carolina

2. Lake Sallie, Minnesota
   Dr. Joe K. Neel, University of North Dakota

3. Diamond Lake, Oregon
   Dr. Howard Horton, Oregon State University

4. Slidell, Texas
   Dr. Dwain Vance, North Texas State University

During the inital site selection tour, contacts and liaison was established by the cooperating investigators with the appropriate government and state agencies at each site to assure their cooperation and to obtain the necessary approvals for the tests to be performed. No problems with state or local approval were encountered.

Field Procedures

Due to the limited amount of test compound available, and the need to contain the test compounds in small areas because of their experimental nature, it was decided to use 60-gallon plastic containment bags as the test vessels at each site, except for the North Texas site, where a whole pond was to be treated.

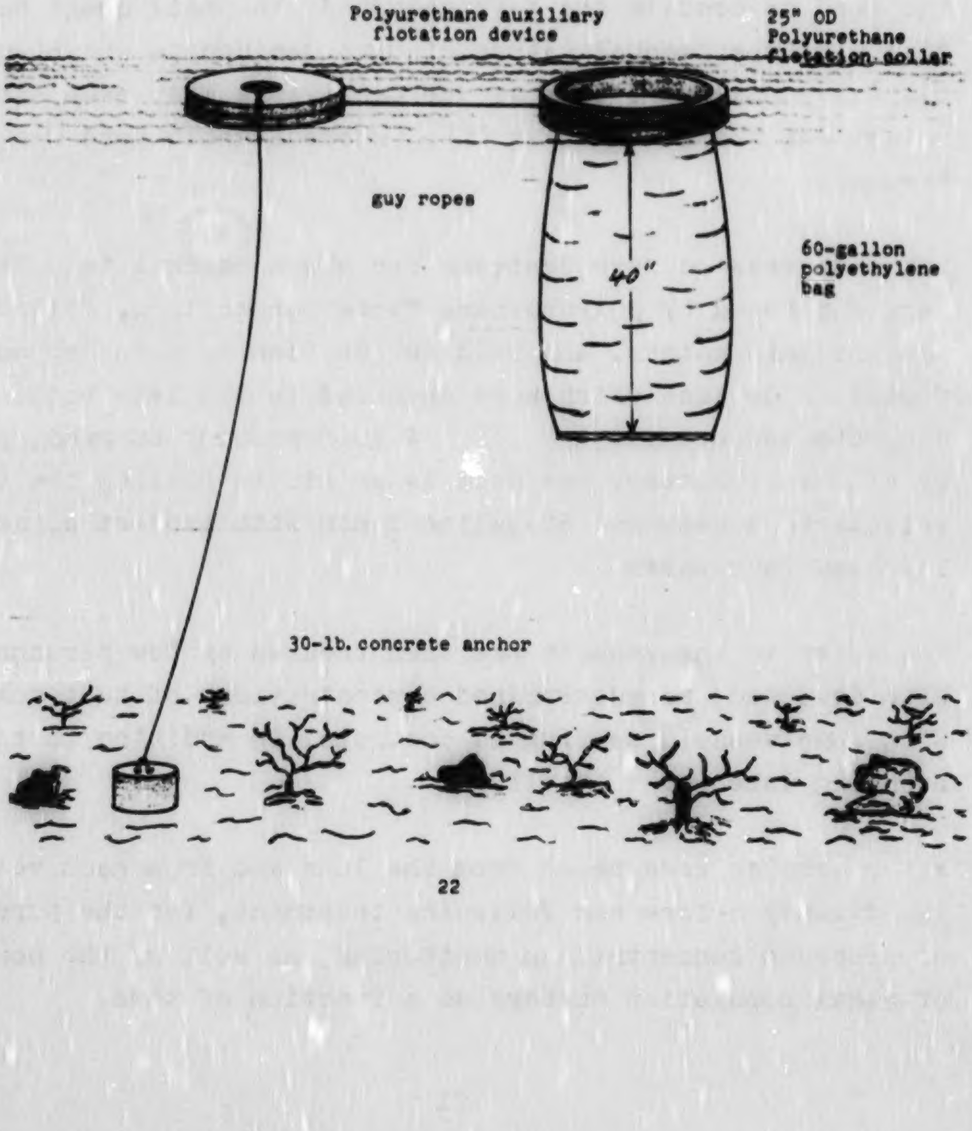
Six test vessels were designed for use at each site. These were supported by polyurethane flotation collars, filled with ambient lake waters, and held in position by means of auxiliary flotation devices which were anchored to the lake bottom with concrete anchors (Figure 1). A self-priming DC pump, powered by a 12-volt battery was used as an aid in filling the test vessels to a measured 60-gallon depth with ambient algae-infested lake water.

The water in the vessels was then treated by Dow personnel with selected, pre-determined concentrations of test compound, with some vessels serving as controls, in addition to the surrounding lake water itself.

Water samples were taken from the lake and from each vessel immediately before and following treatment, for the purposes of compound concentration monitoring, as well as the monitoring of algal population numbers as a function of time.

Figure 1.

OPEN FIELD TEST SYSTEM


Polyurethane auxiliary flotation device
25" OD Polyurethane flotation collar
guy ropes
60-gallon polyethylene bag
40"
30-lb. concrete anchor

Approximately 200 ml samples were taken and placed in plastic biological "twirl pack" bags. Those collected for algal monitoring were treated with formalin solution as preservative measure to assure counting validity. Samples which were taken for compound monitoring were placed in a second protective bag before freezing and were then packed in dry ice for shipment via air freight to Midland, Michigan, for analysis.

Water chemistry data, which varied somewhat with individual preferences of the site investigators, but always included temperature, pH, dissolved oxygen, and alkalinity, were taken before, during, and following treatment according to the recommended schedule.

SAMPLE SHIPMENT

In order to eliminate inconsistencies in sample handling and shipment and to help assure rapid and safe delivery via air freight, the following suggestions were made in a letter sent to each site investigator:

> All samples which are collected for compound analysis and sent to Midland, Michigan, should be:
>
> 1. Sealed securely in primary collection bags (200-300 ml) and then placed in a second protective plastic bag.
>
> 2. Double labeled - (a) pencil writing on a cardboard label, placed inside the second bag.
>   (b) writing on outside or primary collection bag with a permanent-ink felt tipped pen.

3. Uniformly labeled as follows:

I-C	(this is control no. 1, with no test compound)
II-C'	(this is control no. 2, with no test compound)
III-23-1.6	(test compound no. 23 at 1.6 ppm)
IV-23-0.7	(test compound no. 23 at 0.8 ppm)
V-73-1.6	(test compound no. 73 at 1.6 ppm)
VI-73-0.8	(test compound no. 73 at 0.8 ppm)
VII-lake	(ambient lake water outside of test vessel)

4. Quick-frozen as soon as possible after sample collection and retained in a frozen state until packaged for shipment.

5. Packaged in a sturdy, well-insulated shipping carbon, with at least four pounds of dry ice packed around, but protected from, direct contact with the sample bags (utilizing newspapers, or other good packing materials).

6. Package should be labeled "fragile" and sent via air freight by the most direct routing possible using shipping labels which specify the presence of biological samples, packed in dry ice, and a specific request to handle with care, and to deliver on or before a specified date. The only two major air lines which services the Midland, Michigan, area directly are North Central and United.

FORMULATION AND DISPENSING

A rather extensive study was undertaken in order to determine the optimum procedures for formulation of the two compounds. Several solvent systems, balanced to a specific gravity of 1.00 were utilized in the study. Compound No. 23, having a low water solubility, was found to be readily soluble in both acetone and xylene. However, when dissolved in acetone the compound was observed to crystallize and fall out of solution when the concentrate was combined with water in the normal temperature range one would expect to find in lakes and streams. The utilization of xylene as the basic solvent required the addition of about 19 percent perchloroethylene, to yield the desired 1.00 specific gravity balance. The use of a surfactant as an emulsifying agent was also rerequired.

The principal governing factor for the optimum formation of molecular compounds in such cases is the realization of the best hydrophylic-lipophylic balance, permitting the formation of lytotropic mesomorphous phases or dispersion into miscelles. The optimum formulation for Compound No. 23 was determined as a 25:75 ratio of anionic:nonionic surfactant in a xylene-perchloroethylene solvent system.

Compound No. 73 was found to be 0.42 percent soluble in water at room temperature and about 4 to 5 percent soluble in methanol. The increased solubility in methanol was not considered sufficient justification in itself to prompt its use as a solvent, which would tend to escalate the economics of the compound when considered for large-scale use. However, the compound was solubilized in methanol for use in certain portions of some of the field tests to determine whether that particular solvent system would affect the compound's algaecidal activity.

A day or two prior to treatment, primary concentrates of the test compounds were made up in pre-determined aliquots in the laboratory and then taken to the treatment site. For later treatments the procedure was modified by taking unmixed, preweighed quantities of the original compound to the test sites and combining with lake water in brown 500 ml bottles to make a "use" concentrate just prior to treatment in order to avoid the possibility of compound degradation. After the "use" concentrates were prepared they were kept away from direct sunlight as an additional safeguard against possible photodegradation. Also, the tests were not begun until near sundown, for the same reasons.

At the North Texas test site where a whole-pond treatment was undertaken, a "use" concentrate of Compound No. 73 was made by addition of a pre-determined portion of the compound to 500 gallons of water in a commercial sprayer, and continuously agitated for one hour before application. The concentrate was then sprayed onto the surface of the pond just before sundown.

LABORATORY SCREENING TESTS

According to plan, screening tests were to be continued on candidate compounds as they were sent to this laboratory from various sources within Dow and elsewhere. The basic screening procedure was modified slightly from that which was used in the Phase III work (Prows and McIlhenny, 1973). All primary screening tests were run for four days against Anabaena only, at 1.6 ppm and 0.8 ppm. If secondary tests were run they included tests down to 0.4 ppm, or lower if deemed expedient, against both target algal species, Anabaena flos-aquae and Microcystis aeruginosa.

In preparation for the screening tests, stock cultures of Anabaena and Microcystis were grown in 1000 ml flasks, cultured in Gorham's medium under 100 foot-candles cool, white light. Twenty-four hours prior to treatment standard inoculum cultures were made up from the initial stock cultures by placing sufficient algal cells in fresh Gorham's medium to bring the standard inoculum to a relative intensity of 0.20. After 24 hours of acclimation in the new medium, the inoculum was ready for use. At "0-day" for a particular test, 29 ml of standard inoculum was placed in each of the 125 ml culture flasks and then inoculated with 1.0 ml formulated test compound of the appropriate concentration such that the 1:30 dilution factor would reduce the final concentration to the desired levels.

The test vessels and their controls were then kept under a constant illumination of 100 foot-candles, cool white fluorescent lighting, and agitated at 80 excursions per minute in 24°C water bath shakers throughout the four-day test period.

Culture growth in each test flask was monitored by cell counts where appropriate, and by relative intensity readings, taken with an AMINCO fluoromicrophotometer, equipped with a blue mercury-vapor fluorescent lamp for the primary illumination source. Light from the lamp was filtered through a No. 5543 band pass filter before entering the sample. Subsequent light being emitted from the fluorescing chlorophyll molecules thus provided an extremely sensitive method of determining in vitro the relative number of living algal cells remaining, without having to go through a time-consuming chlorophyll extraction procedure.

After substracting the background intensities read for each test sample the final relative intensities compared to those of the control cultures gave a basis for computation of the percent control as exhibited by various concentrations of the test compound in question.

SECTION V

DISCUSSION

The primary research efforts of the Phase III program have been to determine the algal control efficacy of two selected algaecidal compounds. These two compounds, No. 23 (2-Dichloro-3,4-dinitrothiophene) and No. 73 ([p-Chlorophenyl]2-thienyl iodonium chloride) emerged from the Phase II study as prime candidates for field testing because of their effectiveness against two of the commonly troublesome blue-green algal species, Anabaena flos-aquae and Microcystis aeruginosa.

FIELD TEST RESULTS

The four test sites were diversely located to give a good cross-section of climatic conditions. Because each test was monitored by a different group, and because the tests were conducted sequentially there are slight differences in the methods of handling the field data.

The general plan was to include two tests at each site during the 1973 summer and fall seasons. Two tests were made at each site, except for North Texas.

In each case the polyurethane flotation bobs for the enclosure, the flotation collars, and the 60 gallon plastic test bags were prepared at Dow and sent to the area investigators previous to the tests. The complete sets of data collected at each site during each test are included in this report in Appendix B.

Chowan River, North Carolina

The North Carolina test site was located on the west shoreline of the Chowan River, about 20 miles southeast of Ahoske, NC (Figure 2). The Chowan River is about four miles wide at that point. Heavy blue-green algal blooms of *Anabaena*, *Aphanizomenan*, and *Microcystis* had been reported as having occurred in the particular section of the River in previous years, being especially common during the summer of 1972. A fertilizer plant, located about 30 miles upstream is reported to have been responsible for a considerable amount of nutrient input to the river, which may have contributed to optimal "bloom" conditions.

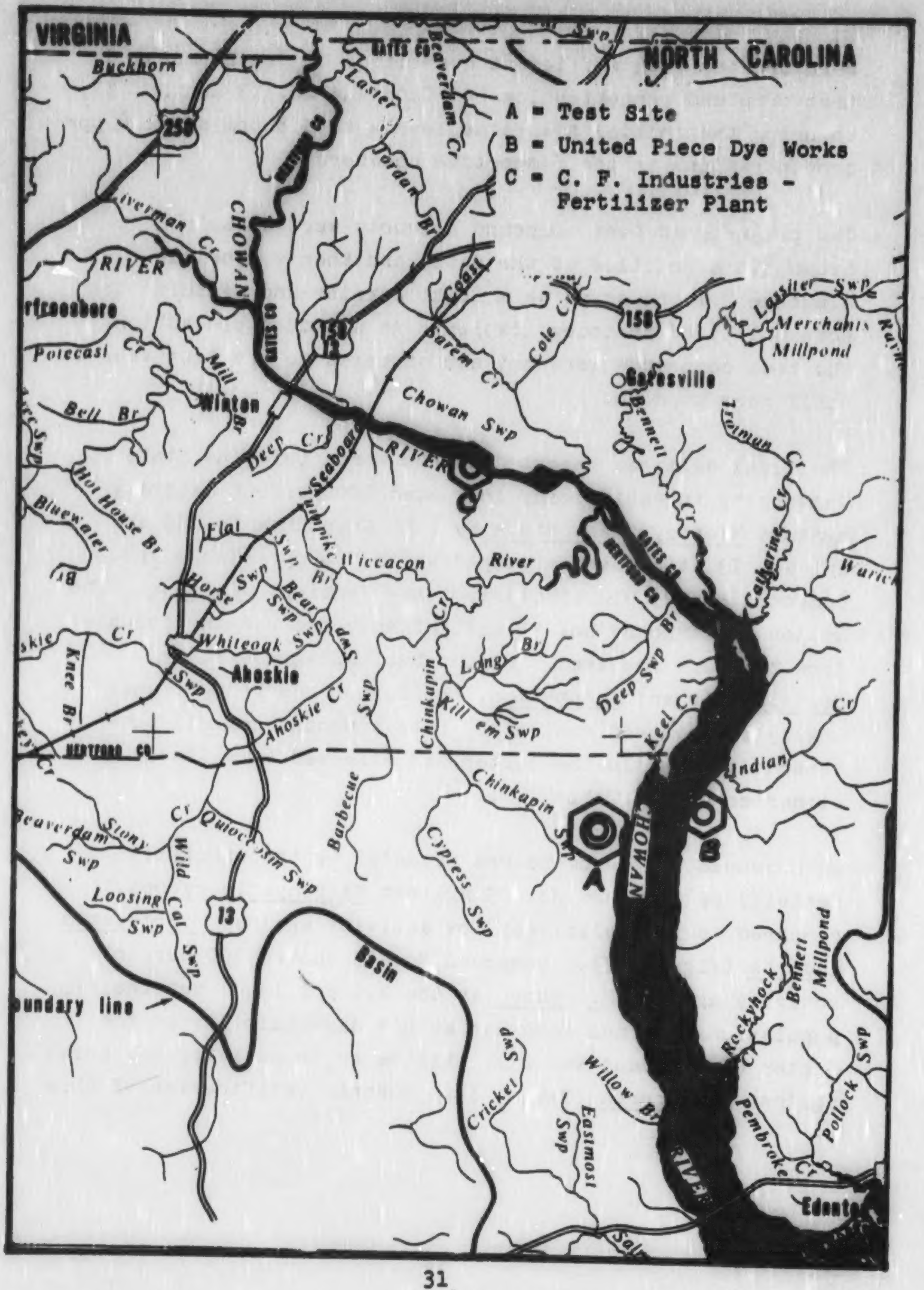
The specific location of the test site was located just to the North of the Perry-Wynn Fish Processing Plant, the owners of which were cooperative and agreeable for the tests to be conducted about 200 yards offshore from their property.

The first test was initiated on June 14, 1973, and a second test was conducted on August 7, 1973, when a much heavier bloom was present.

Specific clearance for conducting tests with experimental compounds was requested and received from the North Carolina State Department of Air and Water Resources by the area investigator, Dr. B. J. Copeland, North Carolina State University.

Of the six 60-gallon test vessels prepared for the field tests, two contained only ambient river water, with no test compound added; to serve as controls, in addition to the surrounding river water itself. The other four vessels

Figure 2. North Carolina Test Site



VIRGINIA
NORTH CAROLINA
A = Test Site
B = United Piece Dye Works
C = C. F. Industries - Fertilizer Plant
Buckhorn
Lassiter
Beaverdam Cr
Jordan Br
CHOWAN
RIVER
Murfreesboro
Potecasi Cr
Mill Br
Winton
Bell Br
Hot House Br
Bluewater Br
Chowan Swp
Cole Cr
Sarem Cr
Gatesville
Bennett Cr
Lassiter Swp
Merchants Millpond
Catherine Cr
Deep Cr
Seaboard
Wiccacon River
Flat Swp
Horse Swp
Bear Swp
Whiteoak Swp
Ahoskie
Ahoskie Cr
Knee Br
Chinkapin Cr
Long Br
Kill em Swp
Deep Swp Br
Keel Cr
Indian Cr
Chinkapin Swp
Cypress Swp
Barbecue Swp
Beaverdam Swp
Stony Cr
Quiocosin Swp
Wild Cat Swp
Loosing Swp
Basin
Boundary line
Cricket Swp
Eastmost Swp
Willow Br
Rockyhock Cr
Bennett Millpond
Pembroke Cr
Pollock Swp
Edenton
258
158
13
A
B
C

were treated with sufficient quantities of pre-formulated test compound concentrations of Compound No. 23 and No. 73, to bring the initial algaecide levels to 0.8 ppm and 1.6 ppm concentrations in the respective chambers.

The pre-weighed test compound aliquots were dissolved in brown 500 ml bottles at the site, and then wrapped in aluminum foil to serve as a light barrier and minimize the possibility of photodegradation. As an added precaution the test compounds were not administered to the test vessels until near sundown.

The algal data, as reported by the North Carolina State University investigators, indicated 100 percent activity against Microcystis montana by both Compounds No. 23 and and No. 73 at concentrations down to 0.8 ppm (Table 1). Compound No. 23 was similarly active against Anabaena. The action of Compound No. 73 against Anabaena was inconclusive from the data received. Of the two blue-green algae, Oscillatoria and Agmenellum, the former was brought under nearly 100 percent control by both compounds at all concentrations, while the latter was affected very little by either compound (Table 2).

An unsuspected phenomena was revealed by the specific activity of Compound No. 23 against Microcystis cyanea, compared to its relatively low activity against Microcystis incerta (Figure 3). Compound No. 73 showed 100 percent activity against M. cyanea at the 1.6 ppm level but the population returned somewhat at 0+4 days (similar to the action of Compound No. 23). Little or no activity was noted against M. incerta (Table 3). Further verification of this

TABLE 1

Chowan River - First Test

Anabaena spp. AND *Microcystis montana*
(cells/liter x 10^6)

Sample	0-days	0+1 day	0+4 days	0+11 days
Ambient River Water	$\frac{7}{15}$	$\frac{28}{11}$	$\frac{33}{4}$	$\frac{5}{0}$
Controls (Avg.)	$\frac{26}{11}$	$\frac{25}{19}$	$\frac{13}{0}$	$\frac{10}{0}$
Compound No. 23 - 0.8 ppm	$\frac{16}{12}$	$\frac{0}{0}$	$\frac{0}{0}$	--
Compound No. 23 - 1.6 ppm	$\frac{10}{12}$	$\frac{1}{0}$	$\frac{1}{0}$	--
Compound No. 73 - 0.8 ppm	$\frac{14}{14}$	$\frac{0}{0}$	$\frac{28}{0}$	$\frac{9}{0}$
Compound No. 73 - 1.6 ppm	$\frac{10}{13}$	$\frac{9}{0}$	$\frac{40}{0}$	$\frac{7}{0}$

KEY: $\frac{\text{Anabaena spp.}}{\text{Microcystis montana}}$

TABLE 2

Chowan River - Second Test

Oscillatoria tenuis AND Agmenellum quadriduplicatum
(cells/liter x 10^6)

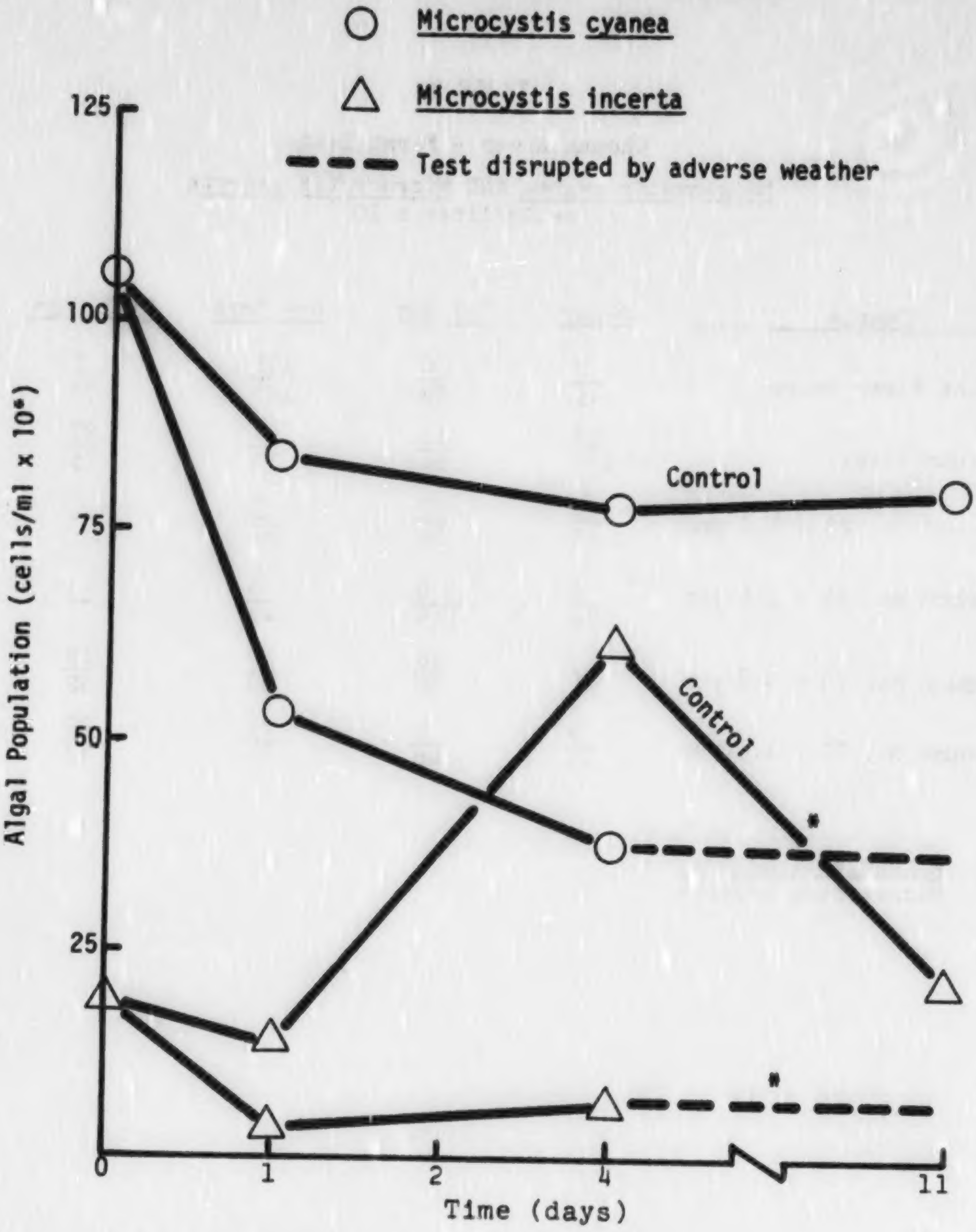
Sample	0-day	0+1 day	0+2 days	0+3 days	0+6 days
Controls	$\frac{16}{93}$	$\frac{?}{?}$	$\frac{18}{9}$	$\frac{?}{?}$	$\frac{46}{13}$
Compound No. 73 - 1.0 ppm	$\frac{16}{106}$	$\frac{0}{94}$	$\frac{4}{38}$	$\frac{0}{84}$	$\frac{9}{102}$
Compound No. 73 - 2.0 ppm	$\frac{16}{98}$	$\frac{0}{26}$	$\frac{10}{35}$	$\frac{14}{70}$	$\frac{12}{74}$
Compound No. 73 - 2.0 ppm*	$\frac{16}{98}$	$\frac{0}{48}$	$\frac{6}{31}$	$\frac{5}{18}$	$\frac{5}{29}$
Compound No. 73 - 3.0 ppm	$\frac{11}{41}$	$\frac{15}{16}$	$\frac{0}{49}$	$\frac{1}{10}$	$\frac{4}{60}$

*Compound solubilized in methyl alchol

KEY: $\frac{\text{Oscillatoria}}{\text{Agmenellum}}$

Figure 3.

Chowan River - First Test
Test Compound No. 23 @ 0.8
ppm vs. M. cyanea & M. incerta



*test disrupted by adverse weather

TABLE 3

Chowan River - First Test

Microcystis cyanea AND *Microcystis incerta*

(cells/liter x 10^{6})

Sample	0-day	0+1 day	0+4 days	0+11 days
Ambient River Water	$\frac{0}{49}$	$\frac{8}{54}$	$\frac{105}{125}$	$\frac{7}{53}$
Controls (Avg.)	$\frac{18}{105}$	$\frac{11}{84}$	$\frac{64}{73}$	$\frac{27}{75}$
Compound No. 23 - 0.8 ppm	$\frac{9}{43}$	$\frac{0}{54}$	$\frac{7}{37}$	--
Compound No. 23 - 1.6 ppm	$\frac{9}{79}$	$\frac{0}{115}$	$\frac{8}{43}$	--
Compound No. 73 - 0.8 ppm	$\frac{9}{85}$	$\frac{16}{36}$	$\frac{10}{89}$	$\frac{19}{52}$
Compound No. 73 - 1.6 ppm	$\frac{9}{45}$	$\frac{0}{46}$	$\frac{21}{85}$	$\frac{29}{73}$

KEY: $\frac{\text{Microcystis cyanea}}{\text{Microcystis incerta}}$

specific activity is seen by examination of the data from the second Chowan test conducted in August. One hundred percent activity was observed against M. cyanea at 2.0 ppm, but little or no significant activity was registered against M. incerta (Figure 4).

The target blue-green alga Anabaena was brought under about 95 percent control by Compound No. 73 at 1.0 ppm during the second Chowan River test (Table 4).

The blue-green algae Oscillatoria planctonica was nearly 100 percent controlled at 0+2 days, but the population returned to normal levels at 0+6 days (Table 5). It is strongly suspected that the population return was the result of a considerable amount of intermixing with river water, which may have occurred during turbulent weather, or from a partially ruptured bag. Oscillatoria tenuis was drastically affected by all four concentrations of Compound No. 73; in contrast, Compound No. 73 showed little or no control of Agmenellum quadruplicatum at the 1.0 ppm level and only partial control at the 2.0 and 3.0 ppm levels (Table 2).

The compound depletion patterns of both test compounds in the Chowan River samples from the two tests were similar (Figure 5). During the second test, after six days, compound No. 73 had been depleted to near or below the 0.1 ppm detection limit in every test vessel (Table 6).

The determined initial compound concentrations in the first test were only about 50 percent (or less) of the calculated values, even though the samples arrived at Dow's Midland, Michigan location still frozen and in good condition (Table 7).

Figure 4.
Chowan River - Second Test
Test Compound No. 73 at 2.0
ppm vs. *M. cyanea* and *M. incerta*

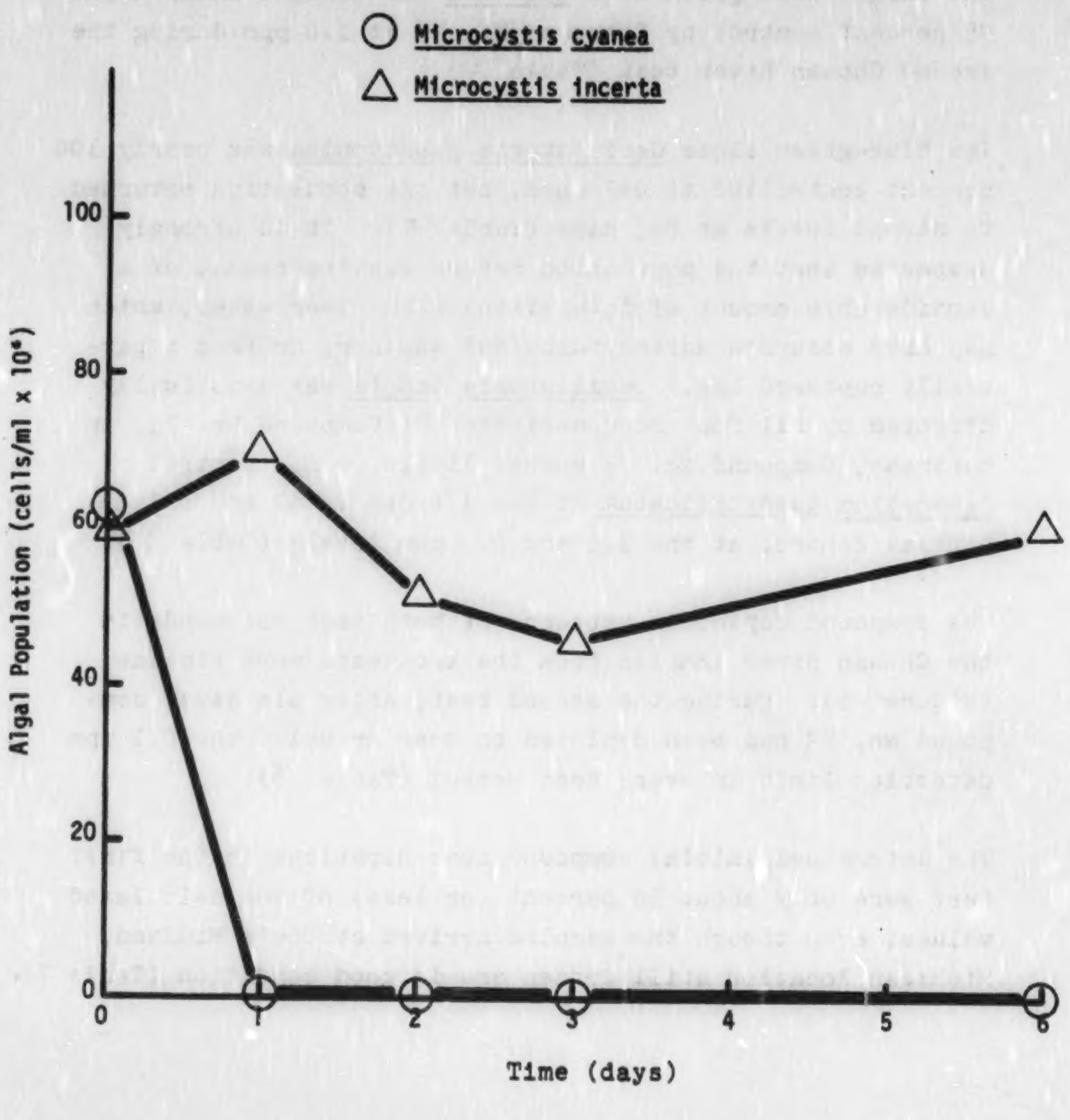


TABLE 4

Chowan River - Second Test

Anabeana spp. AND *Microcystis cyanea*
(cells/liter x 10^6)

Sample	0-day	0+1 day	0+2 days	0+3 days	0+6 days
Controls	$\frac{113}{98}$	$\frac{139}{32}$	$\frac{138}{32}$	$\frac{?}{?}$	$\frac{12}{0}$
Compound No. 73 - 1.0 ppm	$\frac{148}{28}$	$\frac{27}{32}$	$\frac{7}{5}$	$\frac{5}{21}$	$\frac{18}{21}$
Compound No. 73 - 2.0 ppm	$\frac{138}{62}$	$\frac{8}{25}$	$\frac{5}{45}$	$\frac{9}{29}$	$\frac{31}{-}$
Compound No. 73 - 2.0 ppm*	$\frac{138}{63}$	$\frac{5}{0}$	$\frac{2}{0}$	$\frac{6}{0}$	$\frac{10}{0}$
Compound No. 73 - 3.0 ppm	$\frac{109}{60}$	$\frac{9}{0}$	$\frac{7}{0}$	$\frac{11}{2}$	$\frac{20}{0}$

*Compound solubilized in methyl alcohol

KEY: $\frac{\text{Anebaena}}{\text{Microcystis}}$

TABLE 5

Chowan River - Second Test

Oscillatoria planctonica AND Microcystis incerta
(cells/liter x 10^6)

Sample	0-day	0+1 day	0+2 days	0+3 days	0+6 days
Controls	$\frac{101}{40}$	$\frac{?}{-}$	$\frac{141}{31}$	$\frac{?}{?}$	$\frac{62}{73}$
Compound No. 73 - 1.0 ppm	$\frac{108}{98}$	$\frac{14}{97}$	$\frac{3}{62}$	$\frac{25}{21}$	$\frac{81}{66}$
Compound No. 73 - 2.0 ppm	$\frac{62}{62}$	$\frac{0}{25}$	$\frac{0}{44}$	$\frac{0}{28}$	$\frac{50}{112}$
Compound No. 73 - 2.0 ppm*	$\frac{62}{60}$	$\frac{1}{70}$	$\frac{0}{51}$	$\frac{0}{46}$	$\frac{59}{58}$
Compound No. 73 - 3.0 ppm	$\frac{118}{84}$	$\frac{3}{71}$	$\frac{39}{80}$	$\frac{39}{73}$	$\frac{73}{147}$

*Compound solubilized in methyl alcohol

KEY: $\frac{\text{Oscillatoria}}{\text{Microcystis}}$

Figure 5.

Compound Depletion Patterns
Chowan River - First Test

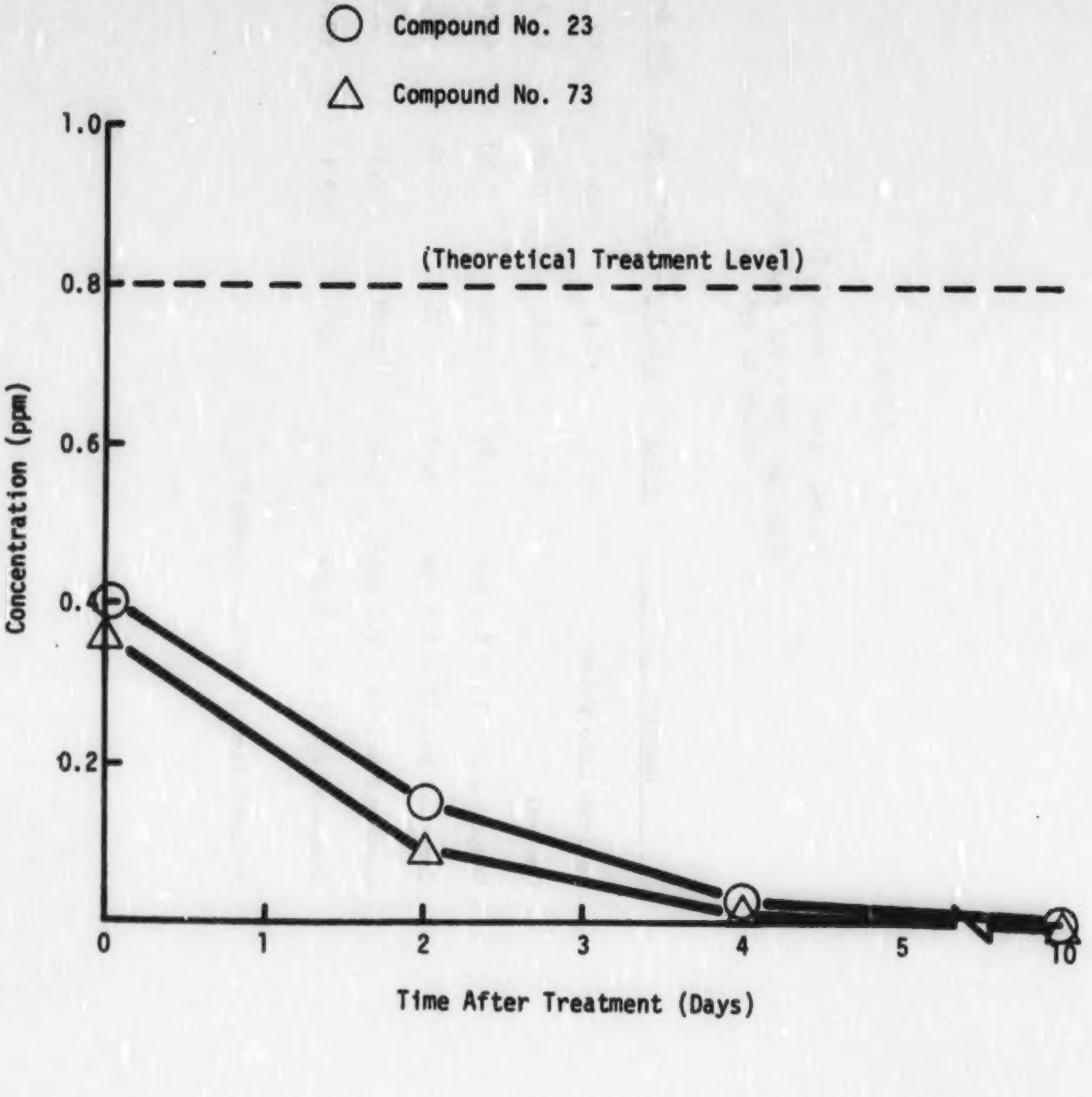


TABLE 6

Chowan River - Second Test

COMPOUND DEPLETION PATTERNS
values in ppm

Sample	0-day	0+1 day	0+2 days	0+3 days	0+6 days
Ambient Lake Water	<0.1	<0.1	<0.1	<0.1	<0.1
Control	<0.1	<0.1	<0.1	<0.1	<0.1
Compound No. 73 - 3.0 ppm	2.85	1.76	0.20	<0.1	<0.1
Compound No. 73 - 2.0 ppm	2.07	0.71	0.34	0.11	0.11
Compound No. 73 - 2.0 ppm*	1.99	0.90	0.33	0.14	0.11
Compound No. 73 - 1.6 ppm	0.92	0.37	<0.1	<0.1	0.11

*Solubilized in methyl alcohol

TABLE 7

Chowan River - First Test

COMPOUND DEPLETION PATTERNS
values in ppm

Sample	0-day	0+2 days	0+3 days	0+4 days	0+6 days	0+11 days
Ambient River Water	0	0		0	0	—
Control Vessel No. 1	<0.05	<0.05				
Control Vessel No. 2	<0.05	<0.05				
Compound No. 23 - 0.8 ppm	0.39	0.15	—	<0.05	—	<0.05
Compound No. 23 - 1.6 ppm	0.39	0.06	—	<0.05	—	<0.05
Compound No. 73 - 0.8 ppm	0.40	0.15	—	<0.05	—	—
Compound No. 73 - 1.6 ppm	0.30	0.10	—	<0.05	—	—

The analysis of samples from the second Chowan treatment showed initial concentrations very close to the calculated values (Table 6), except in the 1.6 ppm test vessel in which the measured concentration was only 0.92 ppm.

The mechanism of degradation is not yet fully understood. Photodegradation is believed to be one of the mechanisms, since a laboratory test run on samples prepared at 1.6 ppm of Compound No. 73 sent to Dow's Midland location for analysis revealed a compound loss of 56 percent on a sample which had been exposed to diffused sunlight for 30 minutes before shipment. This compared to only a 25 percent loss of an identical sample which was not exposed to sunlight (Table 8). The use of plastic bags for sample containers did not prove to be deleterious, as had been previously suspected, but shipping samples in an unfrozen state did cause considerable sample loss. Both glass and plastic-contained, unfrozen samples degraded 19 to 25 percent in nine days without light, which indicates that biodegradation is also involved in the depletion of Compound No. 73.

Water chemistry samples were collected on a regularly scheduled basis during both of the Chowan River tests and the determinations were made either on the site or later at a field test station.

The phosphates were reported as reactive $PO_4^{\equiv}$, total filterable, and total unfilterable phosphates. No particular pattern was seen among the various stations, although somewhat higher levels of phosphates were often, but not always, noted in samples taken from the various test vessels, as compared to ambient river water samples. The NH_4^+ and nitrite-

TABLE 8

Degradation-Absorption Check on Compound No. 73 Under Controlled Conditions

Sample No.	Conditions	Initial Concentration	Final Conc. 0+9 days
I-73-G	in capped glass bottle, unfrozen	1.6 ppm	1.2 ppm
II-73-P	in plastic bag, unfrozen	1.6 ppm	1.3 ppm
III-73-GF	in capped glass bottle, quick frozen in dry ice	1.6 ppm	broken
IV-73-PF	in plastic bag, quick frozen in dry ice	1.6 ppm	1.7 ppm
V-73-GS	in capped glass bottle, previously exposed to open atmosphere for one hour under overcast sky	1.6 ppm	0.7 ppm

nitrogen levels reported were fairly consistant with those in the ambient river water, except in the chambers containing Compound No. 23 where considerably higher levels were noted up to 0+3 days, ranging as high as 11.4 mg/l. The nitrate-nitrogen levels remained higher in the 1.6 ppm tests using Compound No. 23 through 0+4 days. Increased nitrogen concentrations in these vessels were attributed to the nitrogen components in the molecular structure of Compound No. 23, as the most probable source.

The dissolved oxygen levels did not show any significant pattern, and varied from a high of 9.02 ppm in the river at 0-day, to a low of 2.20 ppm at 0+3 days in the test chamber treated with 2.0 ppm of test Compound No. 73, solubilized in methyl alcohol (Table 9). In this vessel, which was added to the experimental design to determine whether such a formulation would enhance algaecidal activity, 361 mg of test compound was dissolved in approximately one pint of methanol before adding to the 60-gallon test chamber. In this procedure the methanol concentration was about 0.2 percent, which is well below the 10,000 ppm methanol toxicity limit for trout. However, the high BOD of methanol did show up significantly in the tests where it was used as a solubilizing agent.

During the first Chowan test the temperature varied from a high of 31.0°C to a low of 26.6°C. However, among the test chambers during any given monitoring period there was little or no difference in temperature and there seemed to be no significant correlation between temperature and test results. The same was generally true of salinity measurements which, for all practical purposes, were below the detection limit throughout both test periods.

TABLE 9

Chowan River - Second Test
DISSOLVED OXYGEN LEVELS

Sample	0-day	0+1 day	0+2 days	0+3 days	0+6 days	0+9 days
Ambient River Water	9.02	8.03	7.52	5.06	6.14	7.29
Control	8.13	8.15	6.94	5.37	5.97	6.41
Compound No. 73 - 3.0 ppm	8.09	6.65	6.24	5.25	7.65	6.55
Compound No. 73 - 2.0 ppm	7.94	6.51	6.12	5.49	7.86	6.85
Compound No. 73 - 2.0 ppm*	7.58	6.44	5.94	2.20	3.28	7.15
Compound No. 73 - 1.0 ppm	8.36	6.63	6.14	5.00	7.74	6.42

*Solubilized in methyl alcohol

Lake Sallie - First Minnesota Test

This test site which was located about five miles southwest of Detroit Lakes, Minnesota, (Figure 6) had been the subject of extensive limnological studies for a number of years, headed primarily by Dr. Joe K. Neel, University of North Dakota who also served as area investigator for this project.

This moderately-sized lake had been in times past very popular for sports fishing, but in recent years had developed serious algal blooms due to heavy nutrient input from sewage lagoons which emptied into Muskrat Lake and which then flowed into Lake Sallie from the east. A state fish hatchery was located at that point between Muskrat Lake (which was used for the second test in that region) and Lake Sallie. Proper liaison was made with the cognizant state agencies and formal approval was received by the area investigators before the test program was begun.

Lake Sallie is about six miles wide, east to west, and about ten miles long. The tests were put into operation at Station 15 across the Lake from the fish hatchery, about 500 yards from the west shore (Figure 7).

The first blue-green algal bloom of the season appeared the first of July. The test compounds were administered in calm and sunny weather to each test chamber, at the calculated 1.6 and 0.8 ppm levels.

Water samples taken before and immediately following test initiation arrived in rather poor condition the fourth day after shipment. Most of the samples were completely thawed, and some of the plastic "twirl pack" bags were ruptured, Fortunately, enough sample in each case remained to allow for fairly accurate determinations to be made, although with some difficulty.

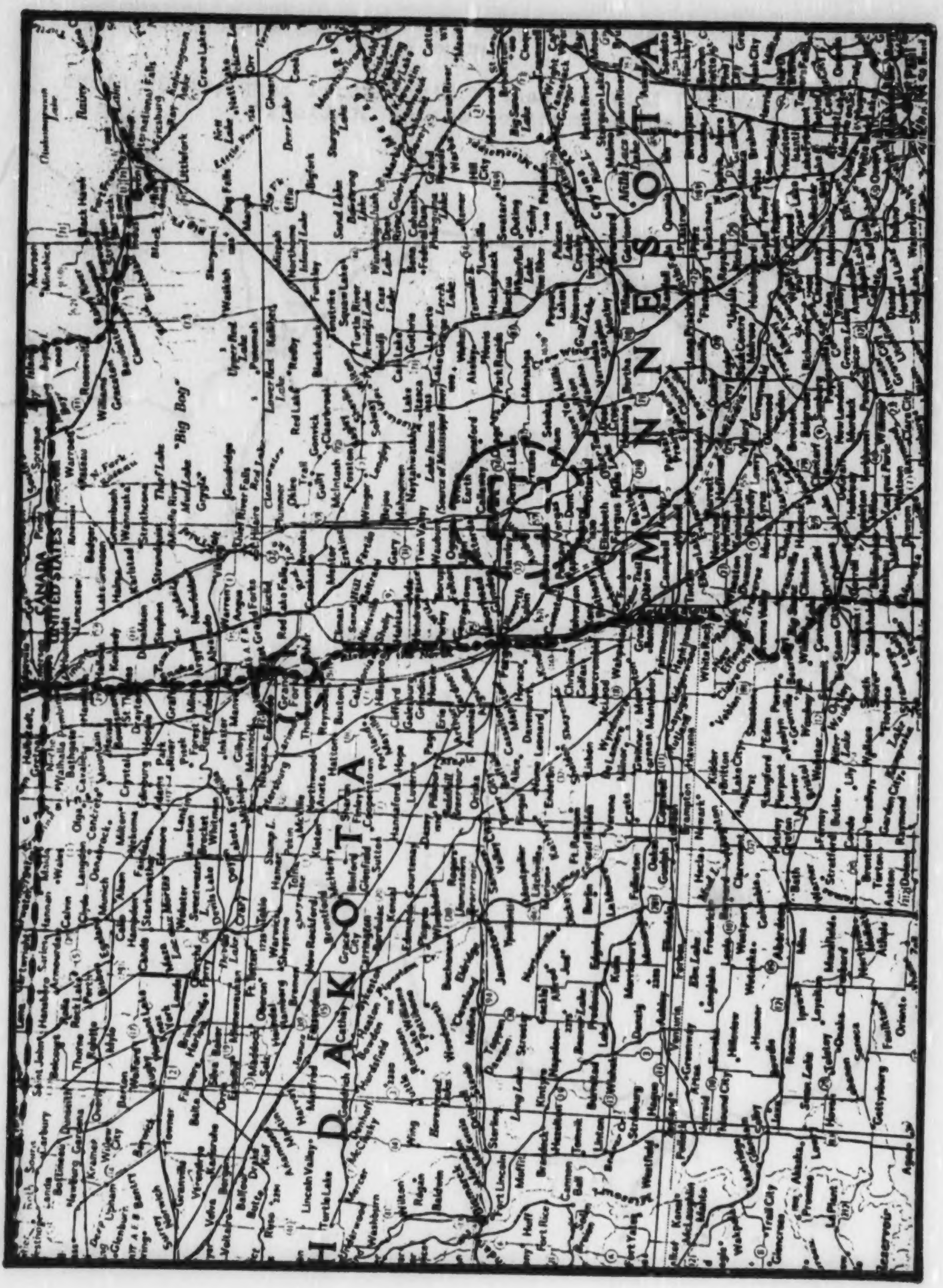
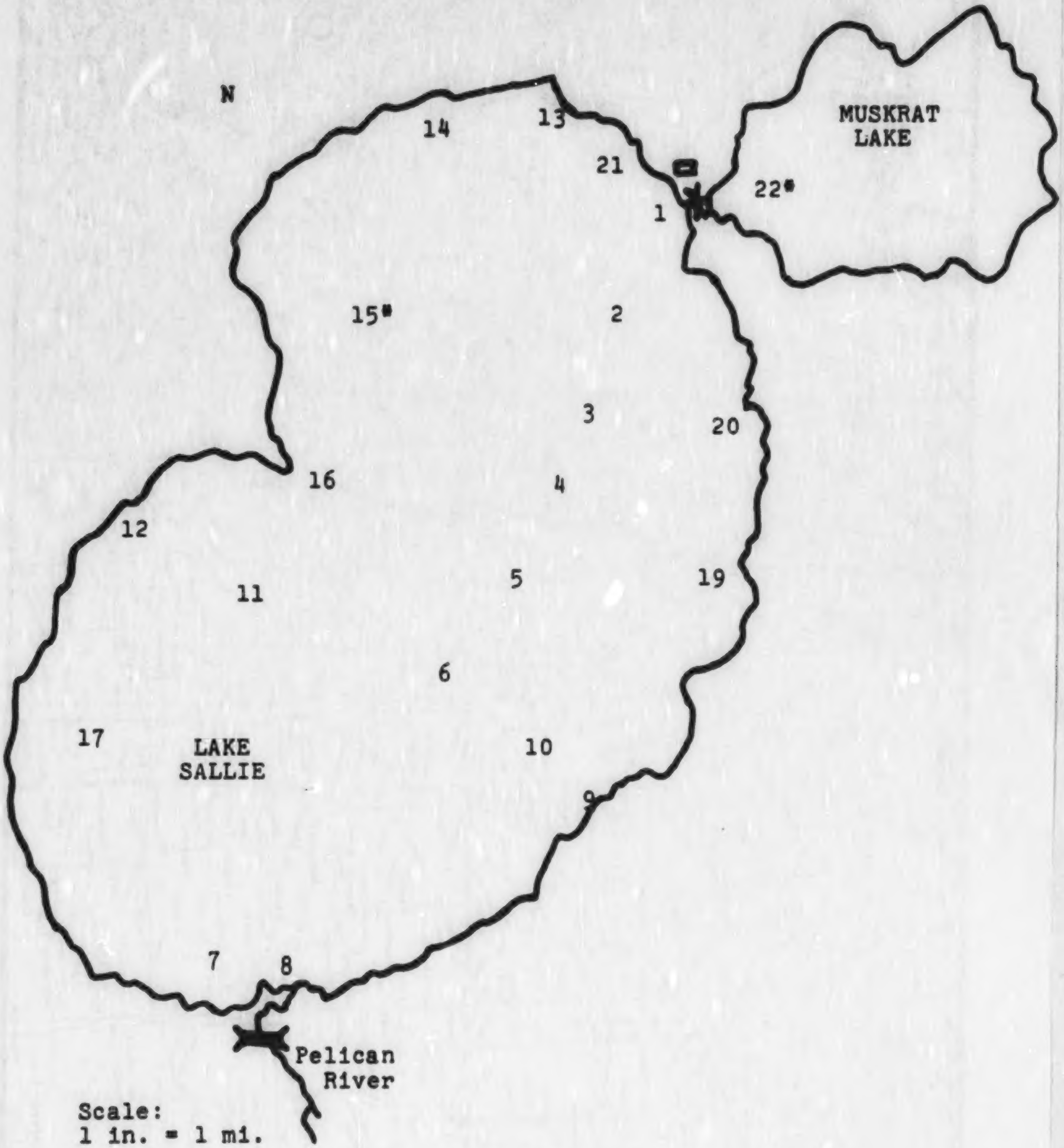

CANADA
UNITED STATES
DAKOTA
MINNESOTA

Figure 6. Minnesota Test Site

Figure 7.

Lake Sallie and Muskrat Lake - Linological Sampling Stations and Algaecide Testing Stations*


N
14
13
21
1
22*
MUSKRAT LAKE
15*
2
3
20
16
4
12
11
5
19
6
17
LAKE SALLIE
10
9
7
8
Pelican River
Scale:
1 in. = 1 mi.

As may be seen from an examination of Table 10 only about 25 to 50 percent of the compound, with which the water was initially treated, was still detectable in the samples at the time they arrived for analysis. Compound No. 23 suffered a greater loss than its companion. The depletion pattern was fairly rapid for both compounds, verifying previous data from the Chowan River tests. The concentration levels of both compounds dropped below the 0.02 ppm and 0.05 ppm detection limits by 0+4 days.

Although variations in available and total PO_4 in the ambient lake water and test vessels were found, no conclusive pattern was discernable. Practically no NO_2^--nitrogen was measured in any of the samples and the NO_3^--nitrogen levels remained rather consistent in the control vessels, compared to the lake water itself, as did the carbonate and bicarbonate levels. The water temperatures were fairly stable, ranging from 23 to 25.5°C, as were the pH readings which ranged from 8.4 to 9.0 during the six-day test period (appendix C).

The algal toxicity of both compounds was immediate and quite effective on Aphanizomenon and three species of Anabaena but it lasted for only two days. The 1.6 ppm concentration of each compound was somewhat more effective than the 0.8 ppm level, achieving 100 percent control of these two species at 0+1 day (Table 11 and Table 12).

Both compounds also affected Coelosphaerium naegelianum through 0+1 days. It can be noted that this blue-green species was not as rapidly affected as were the two filamentous groups and that it did not suffer complete "wipe outs" as they did. The effects on Microcystis were inconclusive from this experiment.

TABLE 10

Lake Sallie - First Test

COMPOUND DEPLETION PATTERNS
values in ppm

Sample	0-days	0+2 days	0+4 days	0+6 days
Ambient Lake Water				
Control Vessel No. 1	--	--	--	--
Control Vessel No. 2	<0.02	--	--	--
Compound No. 23 - 0.8 ppm*	0.39	<0.02	<0.02	<0.02
Compound No. 23 - 1.6 ppm*	0.25	0.04	<0.02	<0.02
Compound No. 73 - 0.8 ppm	0.44	<0.05	<0.05	--
Compound No. 73 - 1.6 ppm	1.2	0.05	<0.05	--

*Compound No. 23 was determined by differential pulse polarography
Compound No. 73 was determined by liquid chromatography

TABLE 11

Lake Sallie - First Test

TEST COMPOUND ACTION ON THE BLUE-GREEN ALGAE

Aphanizomenon AND C. naegelianum

(cells/ml x 10^2)

Sample	0-day	0+1 day	0+2 days	0+4 days	0+6 days
Ambient Lake Water	$\frac{49.5}{81.6}$	$\frac{166}{120}$	$\frac{130}{148}$	$\frac{77.0}{144}$	$\frac{168}{100}$
Control	$\frac{72.6}{81.6}$	$\frac{74.8}{144}$	$\frac{117}{199}$	$\frac{107}{149}$	$\frac{161}{103}$
Compound No. 23 - 0.8 ppm	$\frac{24.2}{163}$	$\frac{0.0}{9.6}$	$\frac{66}{100}$	$\frac{95.7}{170}$	$\frac{126.5}{100}$
Compound No. 23 - 1.6 ppm	$\frac{0.0}{88.8}$	$\frac{0.0}{14.4}$	$\frac{30.8}{76.8}$	$\frac{93.5}{129}$	$\frac{143}{129}$
Compound No. 73 - 0.8 ppm	$\frac{3.3}{136}$	$\frac{2.2}{43.2}$	$\frac{127}{196}$	$\frac{66}{91.2}$	$\frac{156}{48}$
Compound No. 73 - 1.6 ppm	$\frac{0.0}{52.8}$	$\frac{0.0}{19.2}$	$\frac{29.7}{81.6}$	$\frac{67.1}{98.4}$	$\frac{113}{81.6}$

KEY: $\frac{\text{Aphanizomenon}}{\text{C. naegelianum}}$

TABLE 12

Lake Sallie - First Test

TEST COMPOUND ACTION ON THE BLUE-GREEN ALGAE Anabaena AND Microcystis (cells/ml x 10^2)

Sample	0-day	0+1 day	0+2 days	0+4 days	0+6 days
Ambient Lake Water	$\frac{18.7}{81.2}$	$\frac{13.7}{56.}$	$\frac{120}{36.4}$	$\frac{102}{16.8}$	$\frac{186}{16.8}$
Control	$\frac{91.2}{120}$	$\frac{91.2}{81.2}$	$\frac{131}{117}$	$\frac{115}{98}$	$\frac{177}{131.6}$
Compound No. 23 - 0.8 ppm	$\frac{0.87}{95.2}$	$\frac{0.0}{61.6}$	$\frac{58.7}{75.6}$	$\frac{97.5}{53.2}$	$\frac{206}{72.8}$
Compound No. 23 - 1.6 ppm	$\frac{0.0}{47.6}$	$\frac{0.0}{61.6}$	$\frac{32.5}{44.8}$	$\frac{105}{58.8}$	$\frac{198}{61.6}$
Compound No. 73 - 0.8 ppm	$\frac{6.2}{47.6}$	$\frac{5.0}{28.0}$	$\frac{93.7}{30.8}$	$\frac{90.0}{36.4}$	$\frac{151}{67.2}$
Compound No. 73 - 1.6 ppm	$\frac{0.0}{72.8}$	$\frac{0.0}{53.2}$	$\frac{55.0}{36.4}$	$\frac{71.2}{56.0}$	$\frac{155}{106}$

KEY: $\frac{\text{Anabaena}}{\text{Microcystis}}$

Altogether 30 species of algae were identified and enumerated in ambient lake water and in each test vessel (see appendix). The total count of ten species of blue-greens reached a maximum of 59,600 cells/ml at 0+1 day im ambient lake water and a minimum of 6,750 cells/ml with Compounds No. 23 and 73 at the 0.8 ppm level during the same period (Table 13). The total of all algal cells dropped to a low of 29,800 cells/ml in the 1.6 ppm vessel of Compound No.23 at 0+1 day, compared to 81,900 cells/ml in the surrounding lake water (Table 14).

It is also noteworthy that both algaecides are apparently quite specific for blue-greens, with the green alga Oocystis and the diatoms Staurastrum, Fragillaria, Synedra, and Stephanodiscus being affected little or not at all (Table 15).

Muskrat Lake - Second Minnesota Test

The second test in the northern section of the United States was begun on Muskrat Lake, a smaller lake adjacent to Lake Sallie on August 28. Muskrat Lake is smaller and better protected from the wind. The severe wave action had upset some of the test vessels in the previous test, and caused difficult sampling problems. The algae bloom was denser than in Lake Sallie.

The flotation equipment was assembled, anchored into place, and filled with ambient water by mid-afternoon, but the test compound was not administered until near sundown, according to plan, to minimize photodegradation during the initial action period. Only Compound No. 73 was used in this, and all subsequent tests.

TABLE 13

Lake Sallie - First Test

TOTAL BLUE-GREEN ALGAE
(cells/ml x 10^2)

Sample	0-day	0+1 day	0+2 days	0+4 days	0+6 days
Ambient Lake Water	282	597	520	393	596
Control	335	381	573	462	596
Compound No. 23 - 0.8 ppm	227	67.5	310	417	555
Compound No. 23 - 1.6 ppm	111	78	222	423	613
Compound No. 73 - 0.8 ppm	167	67.5	454	285	471
Compound No. 73 - 1.6 ppm	84	79.5	195	288	472

TABLE 14

Lake Sallie - First Test

TOTAL ALGAL CELL COUNTS X 10^2/ml
(includes 30 species of blue-greens, greens,
diatoms, euglenophyta and dinophta)

Sample	0-day	0+1 day	0+2 days	0+4 days	0+6 days
Ambient Lake Water	429	819	—	754	753
Control	526	693	910	852	873
Compound No. 23 - 0.8 ppm	510	348	516	718	792
Compound No. 23 - 1.6 ppm	339	298	411	735	884
Compound No. 73 - 0.8 ppm	352	327	651	524	624
Compound No. 73 - 1.6 ppm	338	360	417	645	610

TABLE 15

Lake Sallie - First Test

TEST COMPOUND ACTION ON
Oocystis and *Fragillaria*
(cells/ml x 10^2)

Sample	0-day	0+1 day	0+2 days	0+4 days	0+6 days
Ambient Lake Water	22.1 44.4	90.1 50.4	93.5 49.2	129.2 4.8	115 7.2
Control	23.8 36.0	71.4 66.0	142 38.4	189 49.2	156 33.6
Compound No. 23 - 0.8 ppm	80.0 56	57.8 70.8	93.5 36.0	171 40.8	134 34.8
Compound No. 23 - 1.6 ppm	62.9 69.6	71.4 61.2	91.8 30.0	163 [illegible].4	170 33.6
Compound No. 73 - 0.8 ppm	28.9 58.8	30.6 67.2	61.2 25.2	113 28.8	88.4 32.4
Compound No. 73 - 1.6 ppm	44.2 58.0	45.9 90.0	62.9 46.8	144 40.8	39.1 27.6

KEY: $\frac{\text{Oocystis}}{\text{Fragilaria}}$

The flotation devices were anchored to bottom anchors and to a small wooden platform at Station 22 (Figure 7), to aid in servicing and collecting samples from the test chambers, rather than from a boat as before. This design seemed to be a considerable improvement and eliminated some of the sampling problems encountered previously during windy weather.

The tests were conducted at the 0.4, 0.8, and 1.6 ppm levels, using only Compound No. 73. Initial monitored compound levels ranged from 63 to 82 percent of the calculated values. The pattern of declining test compound concentrations as a function of elapsed time after initiation of the tests is shown in Figure 8. Compound levels generally dropped below the 0.05 ppm detection limit at 0+6 days, although 0.18 ppm still remained in the 3.2 ppm (initial) vessel at 0+9 days. No compound was detected in the 0.8 ppm vessel at 0+3 days (Table 16).

Algae concentrations in the lake during the test were fairly high, with Oscillatoria being the predominant species and two other blue-greens, Aphamizomenon and Anabaena, ranking second and third (Appendix B).

Anabaena and Aphamizomenon were both drastically affected by the test compound, with no living cells remaining after the first day. No population recovery of these two species was noted within the nine-day monitoring period (Table 17). Raphidiopsis curvata was similarly affected, but the activity against Coelosphaerium naegelianum was variable and inconclusive (Table 18). The test compound registered no appreciable effect against Oscillatoria (Table 19).

Figure 8.

Compound Depletion Patterns
Second Minnesota Test - Muskrat Lake
Test Compound No. 73

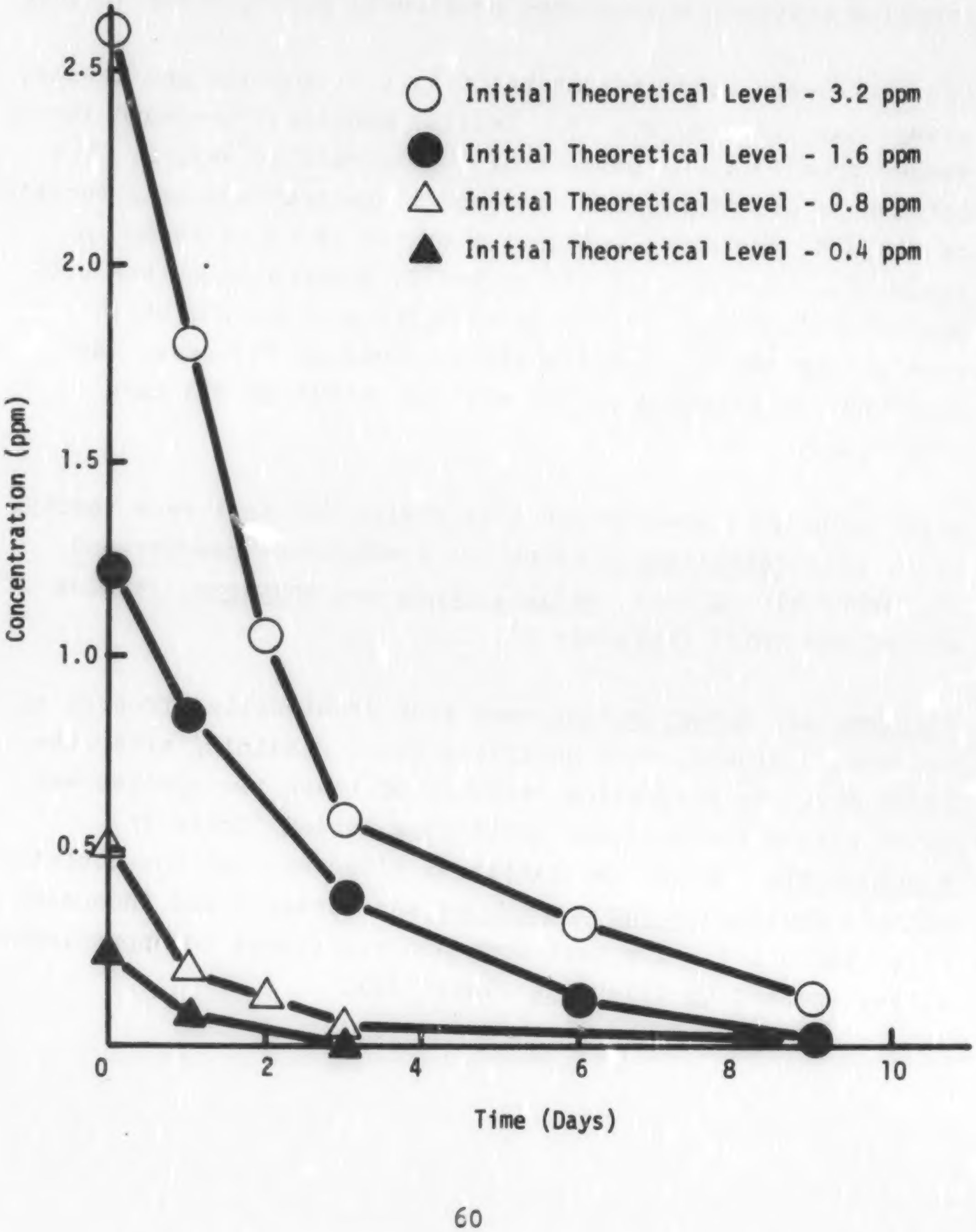


TABLE 16

Muskrat Lake - Second Minnesota Test

Compound Depletion Patterns
values in ppm

Sample	0-day	0+1 day	0+2 days	0+3 days	0+6 days	0+9 days
Control	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Compound No. 73 - 3.2 ppm	2.62	1.86	1.03	0.63	0.37	0.18
Compound No. 73 - 1.6 ppm	1.20	0.86	--	0.39	0.12	<0.05
Compound No. 73 - 0.8 ppm	0.50	0.22	0.14	<0.05	<0.05	<0.05
Compound No. 73 - 0.8 ppm*	0.56	0.31	0.18	0.11	<0.05	<0.05
Compound No. 73 - 0.4 ppm	0.27	0.19	--	0.09	<0.05	<0.05

*Solubilized in methyl alcohol

TABLE 17

Muskrat Lake - Second Minnesota Test

Anabaena AND Aphanizomenon
(cells/ml x 10^2)

Sample	0-day	0+1 day	0+2 days	0+3 days	0+6 days	0+9 days
Ambient Lake Water	$\frac{49.8}{464}$	$\frac{14.1}{381}$	$\frac{56.7}{303}$	$\frac{52}{288}$	$\frac{13.7}{210}$	$\frac{13.6}{190}$
Control	$\frac{46}{43.4}$	$\frac{115}{396}$	$\frac{501}{245}$	$\frac{75.6}{95.8}$	$\frac{69.7}{0}$	$\frac{94.1}{0}$
Compound No. 73 - 0.4 ppm	$\frac{34.1}{504}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$
Compound No. 73 - 0.8 ppm	$\frac{113}{404}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$
Compound No. 73 - 0.8 ppm*	$\frac{5}{252}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{-}{-}$	$\frac{0}{0}$	$\frac{0}{0}$
Compound No. 73 - 1.6 ppm	$\frac{93.2}{262}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$
Compound No. 73 - 3.2 ppm	$\frac{7.62}{218}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$

*Solubilized in methyl alcohol

KEY: $\frac{\text{Anabaena}}{\text{Aphanizomenon}}$

TABLE 18

Muskrat Lake - Second Minnesota Test

Coelosphaerium AND *Raphidiopsis*
(cells/ml x 10^2)

Sample	0-day	0+1 day	0+2 days	0+3 days	0+6 days	0+9 days
Ambient Lake Water	$\frac{108}{61.3}$	$\frac{27.1}{51.7}$	$\frac{23.0}{53.4}$	$\frac{29.9}{72.0}$	$\frac{31.9}{31.8}$	$\frac{31.4}{20.1}$
Control	$\frac{25.2}{6.1}$	$\frac{36.9}{88.7}$	$\frac{89.5}{71.1}$	$\frac{46.3}{66.9}$	$\frac{20.2}{0}$	$\frac{19.4}{0}$
Compound No. 73 - 0.4 ppm	$\frac{56.3}{40.1}$	$\frac{45.8}{0}$	$\frac{0}{0}$	$\frac{6.72}{0}$	$\frac{6.24}{0}$	$\frac{4.32}{0}$
Compound Nc. 73 - 0.8 ppm	$\frac{6.72}{49.1}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{6.48}{0}$	$\frac{0}{0}$	$\frac{0}{0}$
Compound No. 73 - 0.8 ppm*	$\frac{19.2}{14.6}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$
Compound No. 73 - 1.6 ppm	$\frac{7.2}{196}$	$\frac{8.4}{0}$	$\frac{7.2}{0}$	$\frac{0}{0}$	$\frac{11.0}{0}$	$\frac{6.48}{0}$
Compound No. 73 - 3.2 ppm	$\frac{14.6}{106}$	$\frac{0}{0}$	$\frac{7.68}{0}$	$\frac{0}{0}$	$\frac{0.25}{0}$	$\frac{0}{0}$

*Solubilized in methyl alcohol

KEY: $\frac{\text{Coelosphaerium}}{\text{Raphidiopsis}}$

TABLE 19

Muskrat Lake - Second Minnesota Test

Oscillatoria
(cells/ml x 10^{4})

Sample	0-day	0+1 day	0+2 days	0+3 days	0+6 days	0+9 days
Ambient Lake Water	42.7	40.3	39.3	44.2	33.6	39.7
Control	43.5	49.0	35.4	35.4	20.2	24.0
Compound No. 73 - 0.4 ppm	38.8	39.2	27.6	23.6	35.4	26.7
Compound No. 73 - 0.8 ppm	51.3	26.7	39.0	30.4	21.4	26.0
Compound No. 73 - 0.8 ppm*	45.2	27.8	24.7	--	27.1	26.2
Compound No. 73 - 1.6 ppm	31.8	36.2	28.0	28.5	25.1	27.8
Compound No. 73 - 3.2 ppm	39.1	32.8	37.1	26.6	40.7	32.2

*Solubilized in methyl alcohol

The water chemistry data, taken only at "0" and 0+9 days did not reveal any significant patterns of deviation of test vessels from the control values (Appendix B). The temperature dropped 4°C during the nine-day test period. The pH varied from a low of 8.4 in the lake to a high of 9.2 in the 1.6 ppm test chamber at 0+9 days.

Diamond Lake - First Test

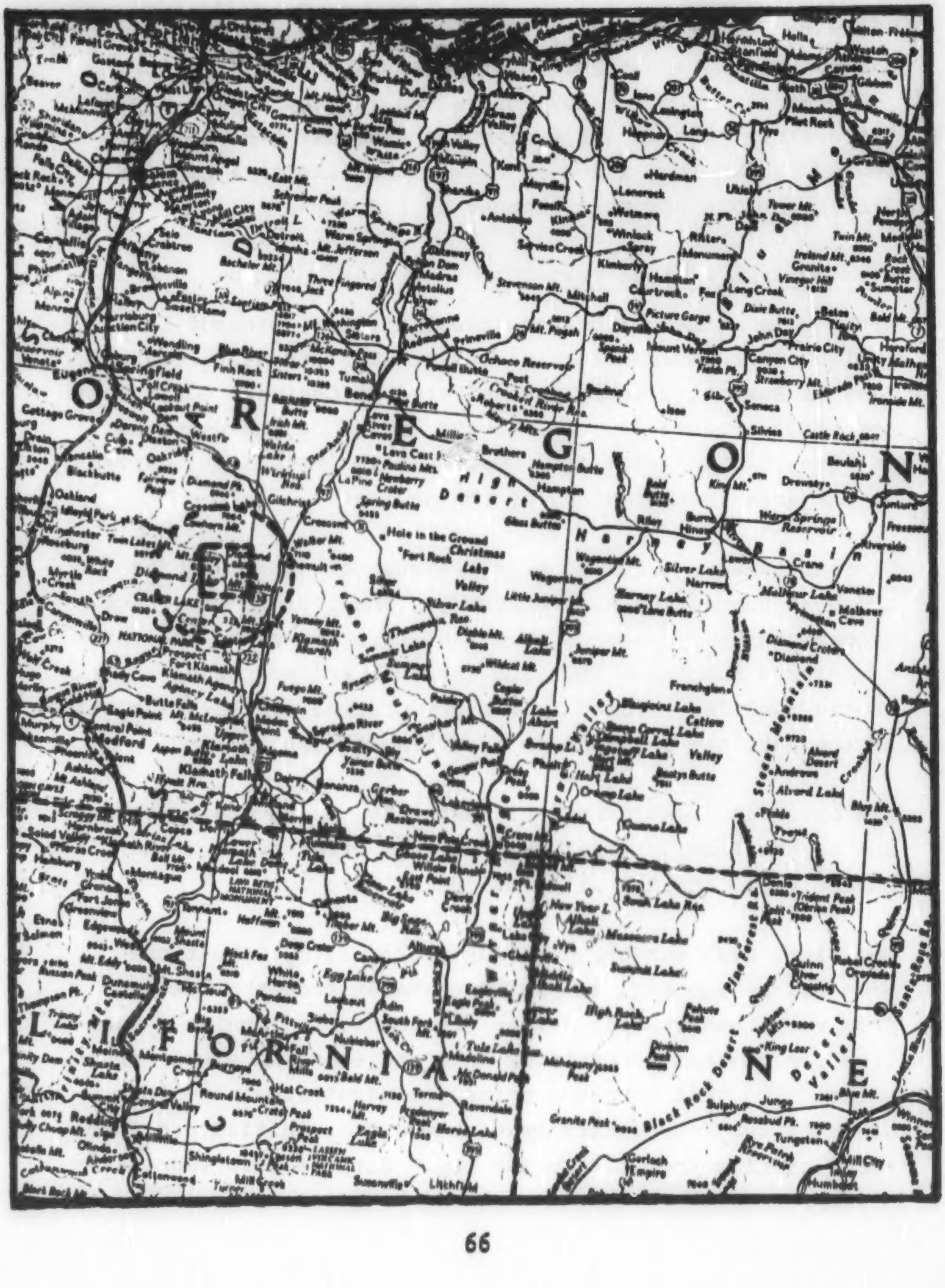
After some rather extensive investigations, Diamond Lake, a high altitude lake located inland about 60 miles southeast of Eugene, Oregon, was selected as the west coast test site (Figure 9). Dr. Howard Horton of the Department of Fisheries and Wildlife, Oregon State University, served as the area investigator for the project.

Diamond Lake is a resort area, famous for trout fishing and both summer and winter sports. The Diamond Lake Lodge campgrounds and numerous summer homes surrounding the Lake contribute nutrients into the lake via ground seepage from septic tank leach lines. In recent years, over-eutrophication of the lake has resulted in troublesome algal blooms.

The specific location of the test site was on the northeast end of the Lake, several hundred feet offshore from the forest guard station at point "B" (Figure 10).

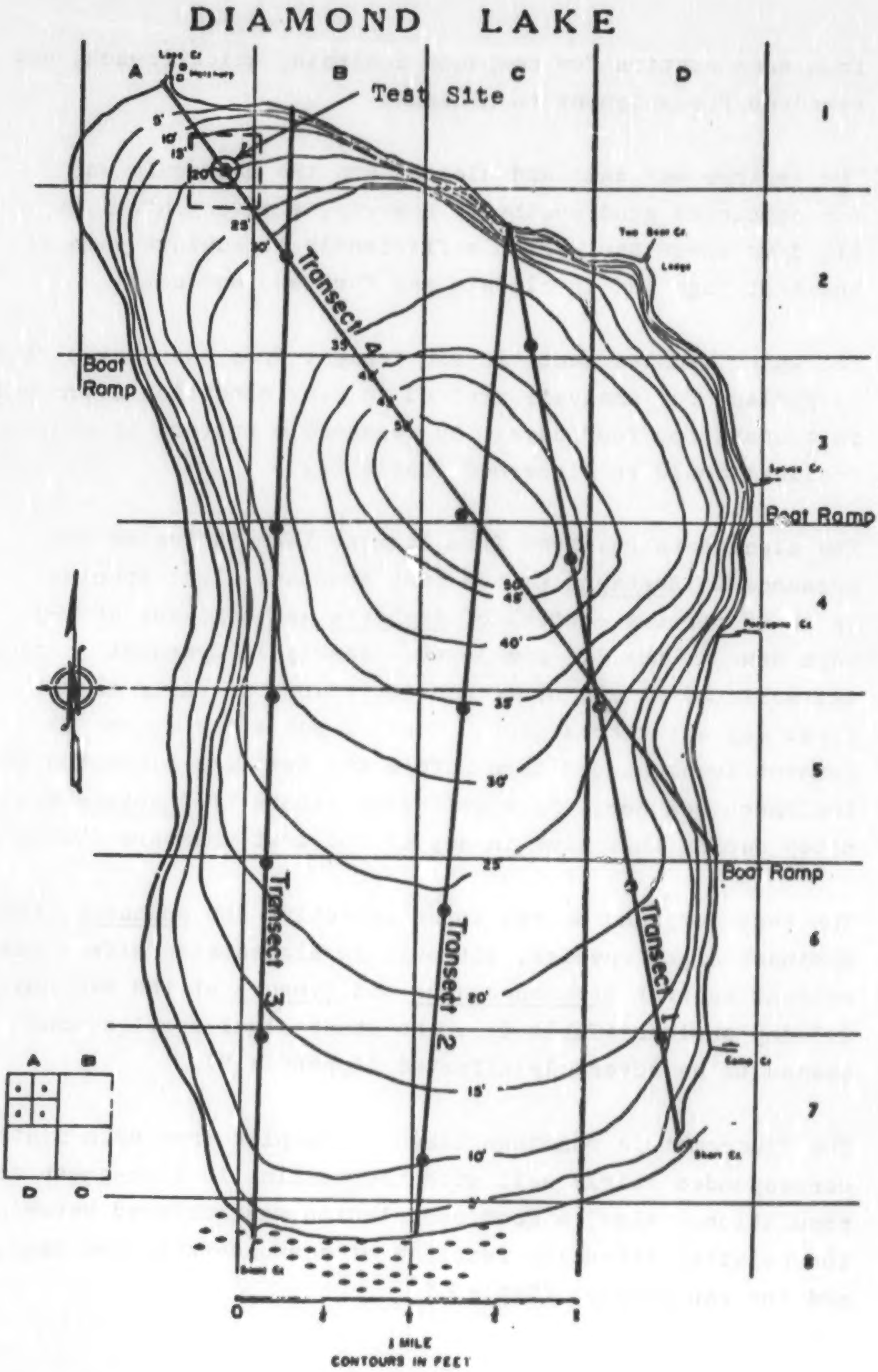
The first test was begun on August 26, 1973. About one hour before sundown, samples were taken from the lake and from each test vessel, before treatment, to furnish a baseline for the water chemistry data and existing algal populations. The test compound primary concentrates were then added to the appropriate test chambers at initial concentrations ranging from 0.4 to 3.2 ppm. Samples were then taken

Figure 9. Oregon Test Site



OREGON
CALIFORNIA
NE
Eugene
Springfield
Cottage Grove
Roseburg
Medford
Ashland
Klamath Falls
Crater Lake
Bend
Prineville
Redmond
La Pine
Gilchrist
Crescent
Fort Rock
Christmas Lake Valley
Silver Lake
Summer Lake
Lakeview
Hole in the Ground
High Desert
Harney Basin
Burns
Hines
Malheur Lake
Harney Lake
Riley
Hampton
Brothers
Lava Cast
Paulina Mt.
Newberry Crater
Spring Butte
Glass Butte
Wagontire
Frenchglen
Steens Mountain
Alvord Lake
Alvord Desert
Diamond Craters
John Day
Canyon City
Prairie City
Seneca
Silvies
Mitchell
Ochoco Reservoir
Three Fingered Jack
Mt. Jefferson
Sisters
Wamic
Maupin
Madras
Tumalo
Alturas
Goose Lake
Tule Lake
Mt. Shasta
Dunsmuir
Redding
Susanville
Black Rock Desert
Denio
Jungo
Sulphur
Gerlach
Empire
Lone Pine
Winnemucca

Figure 10.


DIAMOND LAKE
A
B
C
D
1
2
3
4
5
6
7
8
Test Site
Lodge
Transect 4
Transect 3
Transect 2
Transect 1
Boat Ramp
Boat Ramp
Boat Ramp
5'
10'
15'
20'
25'
30'
35'
40'
45'
50'
1 MILE
CONTOURS IN FEET

from each station for compound analysis, quick frozen, and reserved for shipment to Midland.

The weather was calm and clement and the prognosis was for continued good weather. However, a severe storm at 0+5 days upset the system sufficiently to rupture some of the test bags and invalidate any further test data.

The water samples sent via air freight from the Oregon site to Midland for analysis arrived in poor condition after being in transit for four days. No reasonable pattern of compound residual could be discerned (Table 20).

The algal data received from Diamond Lake indicated the presence of Anabaena as the most abundant algal species. Up to 98 percent control of Anabaena was achieved at 0+3 days down to the 0.4 ppm level. Nearly 100 percent control was achieved at all other concentrations as early as the first day after treatment. Control was still at the 98 percent level at 0+3 days before the test was disrupted by inclement weather. No significant return of Anabaena was noted during this time in any of the test chambers (Table 21).

The test compound seemed to be selective for Anabaena, the dominant algal species, although an algaestatic effect was evident against Stephanodiscus and Synedra at the 0+2 and 0+3 day periods (Table 22). No other algal species monitored seemed to be adversely affected (Appendix B).

The fluorometric readings taken on samples from each station corresponded fairly well with the decline in blue-green algal population. Also, a good correlation was achieved between the relative intensity readings of acetone-extracted samples and the raw samples (Table 23).

TABLE 20

Diamond Lake - First Test
COMPOUND DEPLETION PATTERNS
values in ppm

Sample	0-days	0+1 day	0+2 days	0+3 days
Ambient Lake Water	<0.1	<0.1	<0.1	<0.1
Control Vessel No. 1	<0.1	<0.1	<0.1	<0.1
Compound No. 73 - 0.4 ppm	--	--	—	—
Compound No. 73 - 0.8 ppm	0.67	0.65	0.05	0.0
Compound No. 73 - 0.8 ppm*	0.30	0.57	0.0	0.0
Compound No. 73 - 1.6 ppm	1.07	1.13	0.24	0.13
Compound No. 73 - 3.2 ppm	0.22	0.75	0.20	0.05

*Solubilized in methyl alcohol

TABLE 21

Diamond Lake - First Test
BLUE-GREEN ALGA *Anabaena*
(cells/ml)

Sample	0-days	0+1 day	0+2 days	0+3 days
Ambient Lake Water	4277	4484	3370	2405
Control Vessel No. 1	4144	5984	3207	3312
Compound No. 73 - 0.4 ppm	4336	2189	238	76
Compound No. 73 - 0.8 ppm	5198	0	89	42
Compound No. 73 - 0.8 ppm*	5287	21	0	31
Compound No. 73 - 1.6 ppm	4396	36	74	24
Compound No. 73 - 3.2 ppm	3416	42	0	29

*Solubilized in methyl alcohol

TABLE 22

Diamond Lake - First Test

DIATOMS - Synedra AND Stephanodiscus
(cells/ml)

Sample	0-days	0+1 day	0+2 days	0+3 days
Ambient Lake Water	$\frac{805}{5.6}$	$\frac{1106}{10.1}$	$\frac{1347}{13.1}$	$\frac{1201}{14.9}$
Control Vessel No. 1	$\frac{968}{3.4}$	$\frac{805}{3.8}$	$\frac{2688}{4.0}$	$\frac{5364}{12.4}$
Compound No. 73 - 0.4 ppm	$\frac{1013}{5.9}$	$\frac{843}{4.3}$	$\frac{1013}{3.6}$	$\frac{986}{1.8}$
Compound No. 73 - 0.8 ppm	$\frac{1067}{5.4}$	$\frac{676}{5.2}$	$\frac{966}{3.8}$	$\frac{840}{2.5}$
Compound No. 73 - 0.8 ppm*	$\frac{1180}{7.9}$	$\frac{1094}{6.3}$	$\frac{1267}{6.3}$	$\frac{1213}{4.5}$
Compound No. 73 - 1.6 ppm	$\frac{1019}{7.4}$	$\frac{656}{5.8}$	$\frac{939}{7.2}$	$\frac{900}{3.8}$
Compound No. 73 - 3.2 ppm	$\frac{1213}{6.1}$	$\frac{1019}{3.8}$	$\frac{1115}{6.7}$	$\frac{977}{4.1}$

*Solubilized in methyl alcohol

KEY: $\frac{\text{Synedra}}{\text{Stephanodiscus}}$

TABLE 23

Diamond Lake - First Test
FLUOROMETRIC RELATIVE INTENSITY READINGS

Sample	0-days	0+1 day	0+2 days	0+3 days
Ambient Lake Water	$\frac{0.46}{0.35}$	$\frac{0.41}{0.49}$	$\frac{0.18}{0.15}$	$\frac{0.20}{0.14}$
Control Vessel No. 1	$\frac{0.44}{0.43}$	$\frac{0.41}{0.32}$	$\frac{0.22}{0.19}$	$\frac{0.28}{0.17}$
Compound No. 73 - 0.4 ppm	$\frac{0.53}{0.48}$	$\frac{0.43}{0.45}$	$\frac{0.16}{0.09}$	$\frac{0.15}{0.04}$
Compound No. 73 - 0.8 ppm	$\frac{0.45}{0.35}$	$\frac{0.36}{0.31}$	$\frac{0.05}{0.09}$	$\frac{0.06}{0.04}$
Compound No. 73 - 0.8 ppm*	$\frac{0.56}{0.55}$	$\frac{0.35}{0.42}$	$\frac{0.08}{0.08}$	$\frac{0.05}{0.09}$
Compound No. 73 - 1.6 ppm	$\frac{0.52}{0.44}$	$\frac{0.39}{0.30}$	$\frac{0.12}{0.08}$	$\frac{0.06}{0.06}$
Compound No. 73 - 3.2 ppm	$\frac{0.57}{0.38}$	$\frac{0.25}{0.21}$	$\frac{0.08}{0.06}$	$\frac{0.04}{0.03}$

*Solubilized in methyl alcohol

KEY: $\frac{\text{acetone extraction}}{\text{raw samples}}$

The chlorophyll "a" and carotenoid patterns showed a general decline with increasing time, as compared to the controls, but were not always consistently reduced with increased algaecide concentration, as might have been expected (Table 24).

The temperature in the lake was 16°C at the start of the test, gradually declining to 14°C at 0+3 days. The pH was fairly stable in the 8.9 to 9.1 range. The conductivity of the water did not vary significantly during the three-day test period, but was fairly constant in the 44 to 46 micromhos/cm range. Neither the dissolved oxygen nor the total alkalinity departed significantly from daily control values. The dissolved oxygen increased from a low of 8.1 ppm at the beginning to a high of 9.0 ppm at 0+3 days, primarily because of temperature reduction and increased wind action (see Appendix C).

Diamond Lake - Second Test

The second test at this site was initiated September 17, 1973, following the same basic research plan. A bloom of Anabaena was reported to be persisting, although somewhat reduced. The weather was moderate and fairly calm, although the skies were rather heavily clouded.

The test chambers were all anchored into place and filled with ambient lake water by about 6 pm at which time the test compound aliquots were administered to the respective 60 gallon vessels to yield initial concentrations of 1.0, 0.2, 0.4, 0.8 and 1.6 ppm. One test bag contained only lake water to serve as a comparative control. The lake itself served as a second control.

TABLE 24

Diamond Lake - First Test

CHLOROPHYL "A" AND CAROTENOID LEVELS
(mg/m^3)

Sample	0-days	0+1 day	0+2 days	0+3 days
Ambient Lake Water	11.42 3.04	11.42 3.04	11.42 3.09	11.41 3.04
Control Vessel No. 1	8.81 2.56	11.46 4.05	14.06 3.73	10.43 3.31
Compound No. 73 - 0.4 ppm	9.33 2.56	8.25 2.24	3.56 0.96	2.94 0.75
Compound No. 73 - 0.8 ppm	8.99 1.76	7.59 2.08	2.94 0.69	1.55 0.32
Compound No. 73 - 0.8 ppm*	10.91 2.67	8.08 2.19	2.78 0.80	2.78 1.01
Compound No. 73 - 1.6 ppm	9.84 2.56	7.32 2.03	3.24 1.23	2.89 1.01
Compound No. 73 - 3.2 ppm	10.72 2.83	4.78 2.51	3.94 1.97	1.86 0.59

*Solubilized in methyl alcohol

KEY: Chlorophyl "A" / Carotenoids

The next morning it was evident that a storm front was moving in which increased in severity as the day progressed. The 0+1 day samples were collected in the afternoon with some difficulty due to rain, wind, and high wave action. The storm persisted and increased in severity throughout the night and part of the next day, showing signs of clearing toward evening. However, an examination of the test devices during the afternoon sampling trip revealed that all of the test bags were torn due to pressures caused by violent wave action.

The next day the barometer was up, the weather was calm and intermittently sunny again, and it appeared that the storm front had moved beyond the local area. After some deliberation the decision was made to try to obtain new plastic bags, double them on the flotation collars for added strength, and put the test into operation again, with the hopes of successfully completing the test and obtaining the desired data. The collars had to be cut down to adapt them to the smaller-sized bags which were available. The compound concentrations had to be adjusted accordingly.

The tests were put into operation and the base-line samples were taken as before, completing the last test just as nightfall was approaching. Unfortunately, another storm front moved into the area. But, the storm was less severe and the double bags seemed to hold better.

The monitored initial concentrations agreed fairly well with the calculated values except for samples from Vessel No. IV, in which it appears that the 0-day and the 0+1 day samples may have been inadvertently interposed. The compound concentrations in all test vessels had fallen below the 0.05 ppm detection level at 0+3 days (Table 25) in accordance with previously determined patterns for Compound No. 73.

TABLE 25

Diamond Lake - Second Test
COMPOUND CONCENTRATIONS
ppm

Sample	0-day	0+1 day	0+2 days	0+3 days
Ambient Lake Water	0.05	--*	--*	--*
Compound No. 73 - 4.8 ppm	3.68	3.55	1.12	<0.05
Compound No. 73 - 2.4 ppm	2.24	1.53	0.07	<0.05
Compound No. 73 - 1.2 ppm	1.07	--*	--*	--*
Compound No. 73 - 0.6 ppm	0.15(?)	0.31(?)	<0.05	<0.05
Compound No. 73 - 0.3 ppm	0.25	0.09	<0.05	<0.05

The monitored algal populations, as reported by the Oregon State University investigators, showed that Anabaena was eliminated at all concentrations down to the 0.6 ppm level, within one day after treatment. A fairly large population returned at 0+2 days, however. It is believed that re-inoculation may have occurred due to adverse weather conditions and accompanying heavy wave action on the lake (Table 26). Control Vessel No. 1 and one other vessel were lost early in the test period, as a result of the turbulent weather.

The green alga Gloeocystis was not significantly affected at any test compound concentration used (Table 27). It appears that the desmid, Staurastrum may have been affected initially since the cell count dropped to zero at 0+1 day in the 0.3 ppm and 0.6 ppm vessels and that the vessels were then re-inoculated. The cell count in the 4.8 ppm vessel was only partly reduced however. A similar uncertainty exists with the activity of Compound No. 73 against the diatom, Stephanodiscus (Table 28).

Whole Pond Test - North Texas Site

Small, whole ponds, located near Denton, Texas, were proposed as test sites, in order to duplicate natural circumstances as much as possible.

Several 0.5 to 1.0 acre fishery ponds at the Lewisville State Fish Hatchery had been selected as the test sites of choice, but no suitable blue-green blooms appeared and stabilized during the summer or fall seasons, as had occurred in previous years. Consequently, another pond with an existing bloom in the same general vicinity was chosen. The 0.272-acre farm-pond selected was about 30 miles Northwest of Denton, Texas, (Figure 11) and contained a heavy bloom of blue-green algae.

TABLE 26

Diamond Lake - Second Test

BLUE-GREEN ALGA *Anabaena*
(cells/ml)

Sample	0-days	0+1 day	0+2 days	0+3 days
Ambient Lake Water	124	112	72	24
Control Vessel No. 1	108	68	--	--
Compound No. 73 - 0.3 ppm	188	40	72	68
Compound No. 73 - 0.6 ppm	148	0	40	64
Compound No. 73 - 1.2 ppm	76	--	--	--
Compound No. 73 - 2.4 ppm	64	--	64	104
Compound No. 73 - 4.8 ppm	112	0	0	64

TABLE 27

Diamond Lake - Second Test

GREEN ALGA - *Gloeocystis*
(cells/ml)

Sample	0-days	0+1 day	0+2 days	0+3 days
Ambient Lake Water	40	20	40	52
Control Vessel No. 1	48	0	--	--
Compound No. 73 - 0.3 ppm	48	8	40	32
Compound No. 73 - 0.6 ppm	58	56	36	48
Compound No. 73 - 1.2 ppm	16	--	--	--
Compound No. 73 - 2.4 ppm	44	--	28	32
Compound No. 73 - 4.8 ppm	28	44	36	80

TABLE 28

Diamond Lake - Second Test

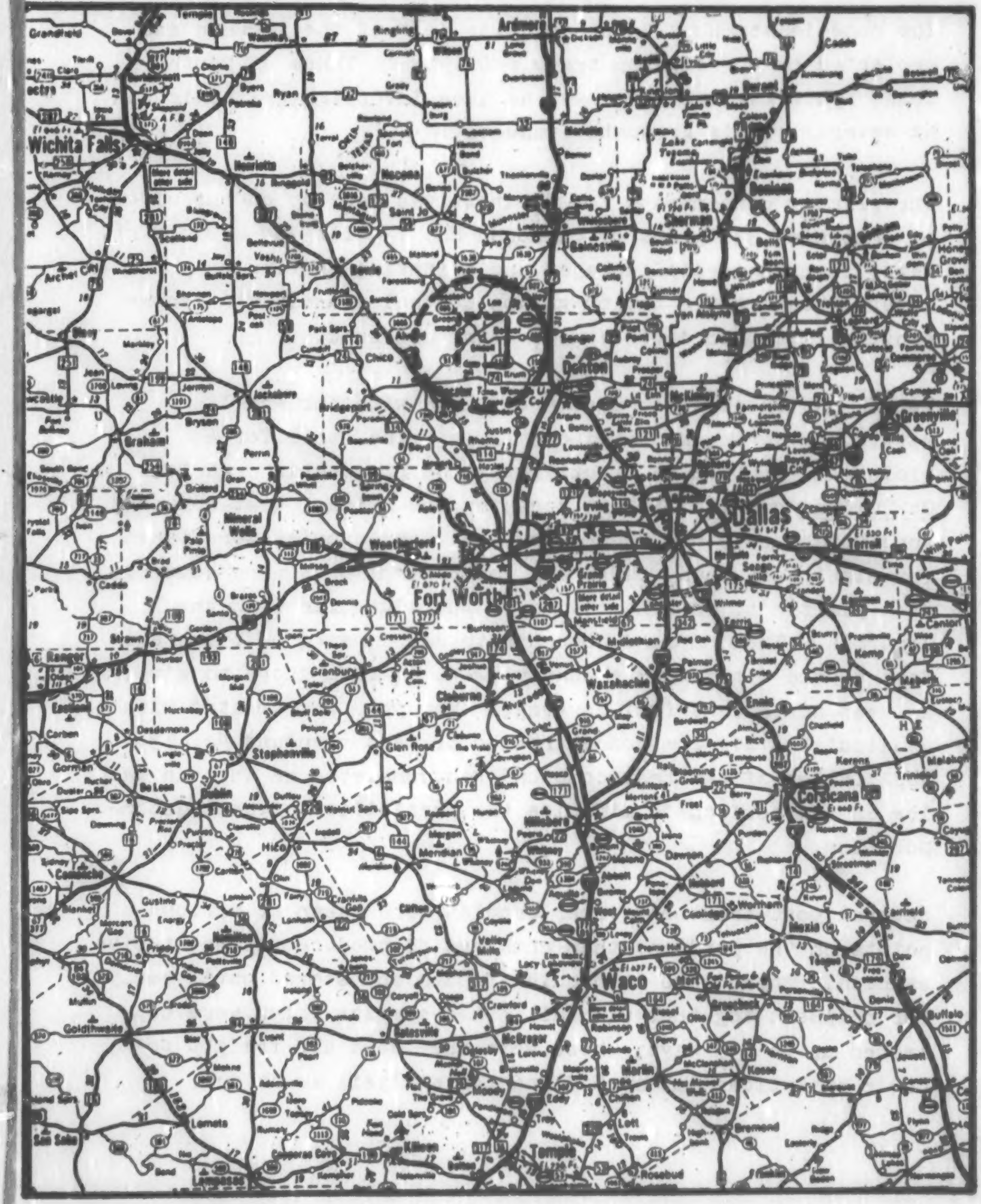
Staurastrum AND Stephanodiscus
(cells/ml)

Samples	0-days	0+1 day	0+2 days	0+3 days
Ambient Lake Water	$\frac{28}{488}$	$\frac{20}{408}$	$\frac{12}{564}$	$\frac{8}{40}$
Control Vessel No. 1	$\frac{4}{68}$	$\frac{4}{156}$	--	--
Compound No. 73 - 0.3 ppm	$\frac{4}{160}$	$\frac{0}{228}$	$\frac{12}{340}$	$\frac{40}{556}$
Compound No. 73 - 0.6 ppm	$\frac{12}{216}$	$\frac{0}{144}$	$\frac{4}{252}$	$\frac{12}{584}$
Compound No. 73 - 1.2 ppm	$\frac{0}{104}$	--	--	--
Compound No. 73 - 2.4 ppm	$\frac{24}{524}$	--	$\frac{12}{536}$	$\frac{16}{356}$
Compound No. 73 - 4.8 ppm	$\frac{16}{172*}$	$\frac{12}{176*}$	$\frac{8}{272*}$	$\frac{12}{328*}$

*Some cells showing signs of degeneration

KEY: $\frac{\text{Staurastrum}}{\text{Stephanodiscus}}$

Figure 11. Northern Texas Test Site


Wichita Falls
Ardmore
Gainesville
Sherman
Denison
Decatur
Denton
McKinney
Greenville
Dallas
Terrell
Fort Worth
Weatherford
Mineral Wells
Graham
Jacksboro
Bowie
Henrietta
Nocona
Saint Jo
Cleburne
Granbury
Stephenville
Glen Rose
Waxahachie
Ennis
Corsicana
Hillsboro
Waco
Mexia
Groesbeck
Marlin
Temple
Killeen
Lampasas
Gatesville
Hamilton
Comanche
Dublin
Hico
Meridian
Clifton
Goldthwaite
San Saba
Eastland
Ranger
Breckenridge
Kerens
Fairfield
Buffalo

The pond is somewhat lower than the rest of the terrain and protected by surrounding trees. Dr. Dwain Vance of North Texas State University served as area investigator, assisted by several of his graduate students.

The primary concentrate for the test was made up on the site by combining 332 grams of Compound No. 73 with 300 gallons of water in a commercial sprayer and agitating continuously for several hours while the 60-gallon control vessels and accompanying flotation devices were being prepared.

Two 60-gallon plastic bags were filled with ambient lake water, one to be used as a control and the other for a biological control test, utilizing the phagocytic organism, Ochromonas ovalis. Before spraying the primary test compound concentrate onto the pond's surface water samples were taken for algal background data, and the control bags were covered with plastic to prevent mixing of the algaecide with the controls. The compound concentrate was sprayed onto the surface of the water from a boat as well as from shore to give an even spread and full coverage. The pond was treated with a calculated 1.0 ppm concentration of test compound No. 73. Application of the test compound was completed as the sun was low in the horizon to minimize the possibility of photodecomposition.

Several post-treatment water samples were taken at various points on the pond and then combined to produce representative samples. These were then quick-frozen and stored until ready for shipment together with all other samples which were collected up to 0+3 days; these were then sent air freight to Dow's analytical lab at Midland for analysis as before.

The analytical data obtained showed an initial concentration of 0.42 ppm, less than one-half of the calculated value, immediately following treatment. Rapid adsorption, due to the high amount of biomass and possible compound biodegradation may have been responsible for the low initial levels detected.

A heavy storm moved into the area on the evening of the first day after treatment, during which time three-inches of rainfall was recorded. The water analysis data showed the compound concentration in the pond at this time to have dropped below the 0.05 ppm detection limit. None of the control samples or subsequent pond samples registered any detectable amounts of the test compound (Table 29).

The algal densities in the test pond, as determined by the University investigators, were very high, ranging up to 250,000 cells/ml in the case of _Agmenellum quadruplicatum_ the dominant species. _Microcystis_ and _Oscillatoria_ ranged from 10 to 60,0000 cells/ml; _Anabaena_ was also present to a lesser extent.

The results of the whole-pond test were not entirely conclusive. Although the test compound level at 0+1 day fell below the 0.05 ppm detection limit, _Oscillatoria_ in the pond was reduced to "0" count from 0+1 day through the end of the 17-day test period. However, _Oscillatoria_ cell counts were noted, intermittently, in only one of the control vessels during this same period. _Agmenellum quadruplicatum_ counts showed a marked reduction from an initial count of 250,000 cells/ml to 17,000 cells/ml at 0+17 days, but no _Agmenellum_ cells were found in samples taken from the control chambers after the first day (Table 30).

TABLE 29

North Texas Test Site

MONITORED TEST COMPOUND LEVELS
Compound No. 73
(ppm)

Sample	0-day	0+1 day	0+2 days	0+3 days	0+6 days	0+17 days
Pond*	0.42	<0.005	<0.005	<0.005	<0.005	<0.005
Control Vessel No. 1	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Control Vessel No. 2	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005

*Initial calculated concentration = 1.0 ppm

TABLE 30

North Texas - Whole Pond Test

Oscillatoria AND Agmenellum
(cells/ml x 10^3)

Sample	0-day	0+1 day	0+2 days	0+3 days	0+6 days	0+17 days
Ambient Pond Water*	$\frac{60}{250}$	$\frac{0}{72}$	$\frac{0}{111}$	$\frac{0}{131}$	$\frac{0}{20}$	$\frac{0}{17}$
Control Vessel No. 1	$\frac{60}{250}$	$\frac{4}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{5}{0}$	$\frac{--}{--}$
Control Vessel No. 2	$\frac{60}{250}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{--}{--}$

*Treated at 1.0 ppm

KEY: $\frac{\text{Oscillatoria}}{\text{Agmenellum}}$

Microcystis cell counts in the pond varied greatly, from 10,000 cells/ml initially to a maximum 150,000 cells/ml at 0+1 day, to "0" from 0+6 days on to the end of the 17-day test period. At the same time, the controls dropped from 10,000 cells/ml to "0" at 0+2 days, with no return of this species (Figure 12). No Anabaena cells were detected after the first day following treatment, until the last samples collected at 0+17 days, in which a heavy bloom of 200,000 cells/ml was found (Table 31).

This observed phenomena shows an apparent shift in biological equilibrium dynamics due to competition reduction but it causes difficulties in assessing the algaecidal activity of Compound No. 73 in a whole pond.

Due to the inconclusive results from the whole-pond test it was especially desirable to conduct a second test of the same type. But the season was already well advanced at this time, and further attempts to find a suitable pond with a naturally occurring blue-green algal bloom was unsuccessful.

ALGAL CONTROL

As one of the primary objectives of the Phase III effort the algal control efficacy of the prime candidate compounds under natural "bloom" conditions was to be determined.

Data collected under widely varied conditions in geographically diverse regions of the United States show a rather distinctive pattern of selective blue-green algal control.

As may be seen by examination of Table 32 and Table 33, good control was exerted over most of the blue-green algae by both test compounds used at each site, with the exception of Microcystis incerta, and under certain conditions

Figure 12.

Whole-Pond Test - North Texas
Compound No. 73 at 1.0 ppm Vs. Microcystis

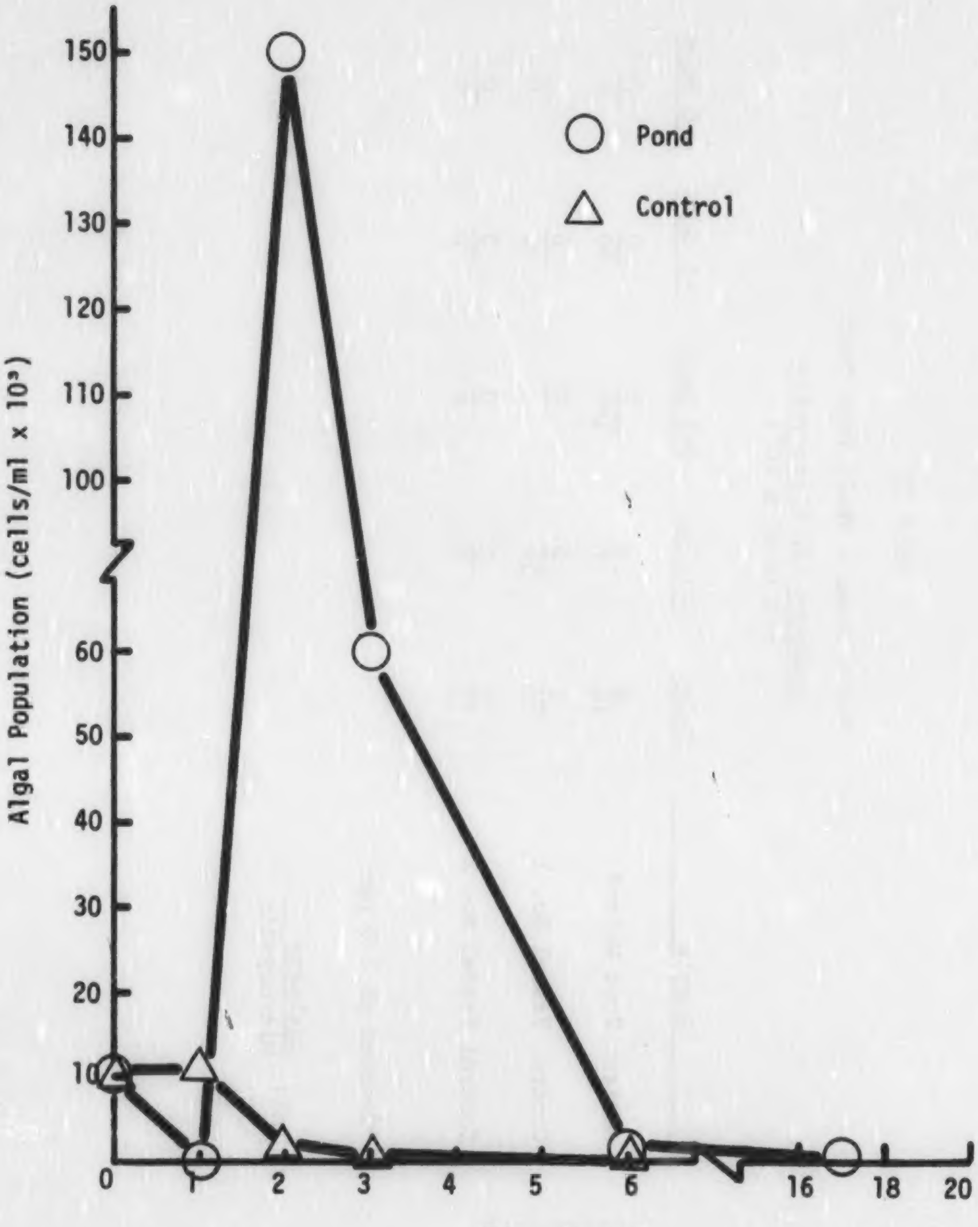


TABLE 31

North Texas - Whole Pond Test

Anabaena AND Microcystis
(cells/ml x 10^3)

Sample	0-day	0+1 day	0+2 days	0+3 days	0+6 days	0+17 days
Ambient Pond Water*	$\frac{1}{10}$	$\frac{0}{0}$	$\frac{0}{150}$	$\frac{0}{60}$	$\frac{0}{0}$	$\frac{200}{0}$
Control Vessel No. 1	$\frac{1}{10}$	$\frac{0}{10}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{-}{-}$
Control Vessel No. 2	$\frac{1}{10}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{-}{-}$

*Treated at 1.0 ppm

KEY: $\frac{\text{Anabaena}}{\text{Microcystis}}$

TABLE 32

PHASE III FIELD TEST RESULTS

Algal Control - Compound No. 73

Species	Chowan River	Sallie	Muskrat	Diamond Lake	Slidell Pond
BLUE-GREENS					
Anabaena	2	1*	1	1 1*	1*
Microcystis spp.		3		1	1
M. cyanea	1	-		-	-
M. incerta	3	-		-	-
Agmenellum	3	-		-	1
Oscillatoria	1	3		-	1
Coelosphaerium	-	2		-	-
Raphidiopsis	-	2	1	-	-
Aphanizomenon	-	1	1	-	-
GREENS					
Gloeocystis	-	-		3	-
Oocystis	-	3		-	-
DIATOMS					
Staurastrum	-	-		3	-
Fragillaria	-	3		-	-
Synedra	-	-		2	-
Stephanodiscus	-	-		2	-

KEY:
1 = good control (80-100%)
2 = moderate control (40-80%)
3 = little or no control (0-40%)

NOTE: double listings is indicative of varied results between the first and second tests

*population return observed before end of test period

TABLE 33

PHASE III FIELD TEST RESULTS

Algal Control - Compound No. 23

Species	N. Carolina Chowan River	Minnesota Sallie & Muskrat	Oregon Diamond Lake	N. Texas Slidell Pond
BLUE-GREENS				
Anabaena	1*	1	--	--
Microcystis spp.	1	2	--	--
M. cyanea	1	-	--	--
M. incerta	2	-	--	--
Agmenellum	3	-	--	--
Oscillatoria	1*	-	--	--
Aphanizomenon	-	1	--	--
GREENS				
Gloeocystis	-	-	--	--
Oocystis	-	3	--	--
DIATOMS				
Staurastrum	-	-	--	--
Fragillaria	-	3	--	--
Synedra	-	-	--	--
Stephanodiscus	-	-	--	--

KEY: 1 = good control (80-100%)
2 = moderate control (40-80%)
3 = little or no control (0-40%)

*population return observed later in test period

Oscillatoria, Coelosphaerium, and Agmenellum quadruplicatum. Since variable results were obtained on the control of these latter four species, it is suggested that this may have been due to a difference in algal strains, which were not differentiated by the university investigators in the reported data. Those results which were somewhat inconclusive, from the available experimental evidence, are indicated with a question mark. Population returns of Anabaena were noted in a number of cases, but it is possible that this may have been due to re-contamination of the vest vessels during turbulent weather conditions. Good control of Microcystis, as well as Anabaena was achieved at all sites except in Minnesota. Raphidiopsis and Aphanizomenon were controlled well by both test compounds at each site where these species were found to be present at the time of treatment.

In no case was either the green algae or diatoms affected significantly by either test Compound No. 23 or No. 73, thus verifying the algal control specificity of the algaecides in question.

TOXICOLOGY

Candidate test compound No. 23 and No. 73 were submitted to Dow's Waste Control Group for preliminary ranging, and subsequent full-scale toxicity tests.

The preliminary ranging tests on Compound No. 23 indicated a fish toxicity level which was too high to allow its continued consideration for use as an algaecide where it would be administered directly to natural water supplies. One hundred percent kills were noted at concentrations down to 0.1 ppm, with a partial kill at 0.065 ppm within the first 24 hours (Table 34). Consequently, Compound No. 23 was withdrawn from the list of candidate test compounds.

TABLE 34

FISH TOXICITY TESTS - BLUE GILLS

Compound No. 23
2,5-Dichloro-3,4-dinitrothiophene

Concentration	24-Hour Toxicity Effect
0.8 ppm	100% kill
0.1 ppm	100% kill
0.065 ppm	partial kill
0.037 ppm	no kill

Compound No. 73, as tested against both blue gills and rainbow trout, proved to be safe to fish life when used in the recommended ranges below 10 ppm. The highest toxicity on blue gills was reported from the 48-hour test, where the lethal concentration to 90 percent of the fish (LC_{90}) showed an average value of 177 ppm (Table 35 and Figure 13). The minimum average lethal concentration to 90 percent of the rainbow trout (LC_{90}) was 209 ppm at 72 hours (Table 36).

LABORATORY SCREENING

A continuing portion of the algaecide development program included routine testing of additional compounds.

During Phase III 70 new compounds were tested for algaecidal activity by the previously established primary screening test procedures. Some of these were re-tests on a few of the more promising Phase II compounds, with new formulations. All were tested against Anabaena flos-aquae at 0.8 ppm. Eight were tested at higher and lower concentrations, ranging from 1.6 ppm down to 0.2 ppm (Table 37). Three were subjected to tests against both Microcystis aeruginosa and Anabaena flos-aquae for greater sensitivity delineation (Table 38).

Five of these compounds tested exceeded the 90 percent control level against Anabaena within the four-day test period. Only two: No. 136, 2,2'-(1,2-Ethylenediyl)bisbenzoxazole and No. 176, 1,2-Dichloro-4-(isothiocyanatomethoxy)benzene were considered to be of continuing interest when other known factors such as compound instability, toxicity potential, manufacturing difficulties, and economics were taken into consideration.

Compound No. 136 showed 91 percent activity against Anabaena at the 0.8 ppm level and exhibited 94 percent control of

TABLE 35

FISH TOXICITY TESTS - BLUE GILLS

Compound No. 73

p-Chlorophenyl-2-thienyl iodonium chloride

24 HOUR TLM*, ppm

	Range		Average
Test	Lower Limit	Upper Limit	Values
LC_{10}	61	180	105
LC_{50}	140	172	155
LC_{90}	131	389	226

48 HOUR TLM*, ppm

	Range		Average
Test	Lower Limit	Upper Limit	Values
LC_{10}	72	141	101
LC_{50}	124	145	134
LC_{90}	126	248	177

72 HOUR TLM*, ppm

	Range		Average
Test	Lower Limit	Upper Limit	Values
LC_{10}	35	165	76
LC_{50}	107	137	121
LC_{90}	89	417	192

96 HOUR TLM*, ppm

	Range		Average
Test	Lower Limit	Upper Limit	Values
LC_{10}	45	137	78
LC_{50}	85	155	115
LC_{90}	96	295	169

*Tolerance Limit with 95% confidence limits

Figure 13.

Fish Toxicity Tests - Blue-Gills
Test Compound No. 73 - 48 hour TLM

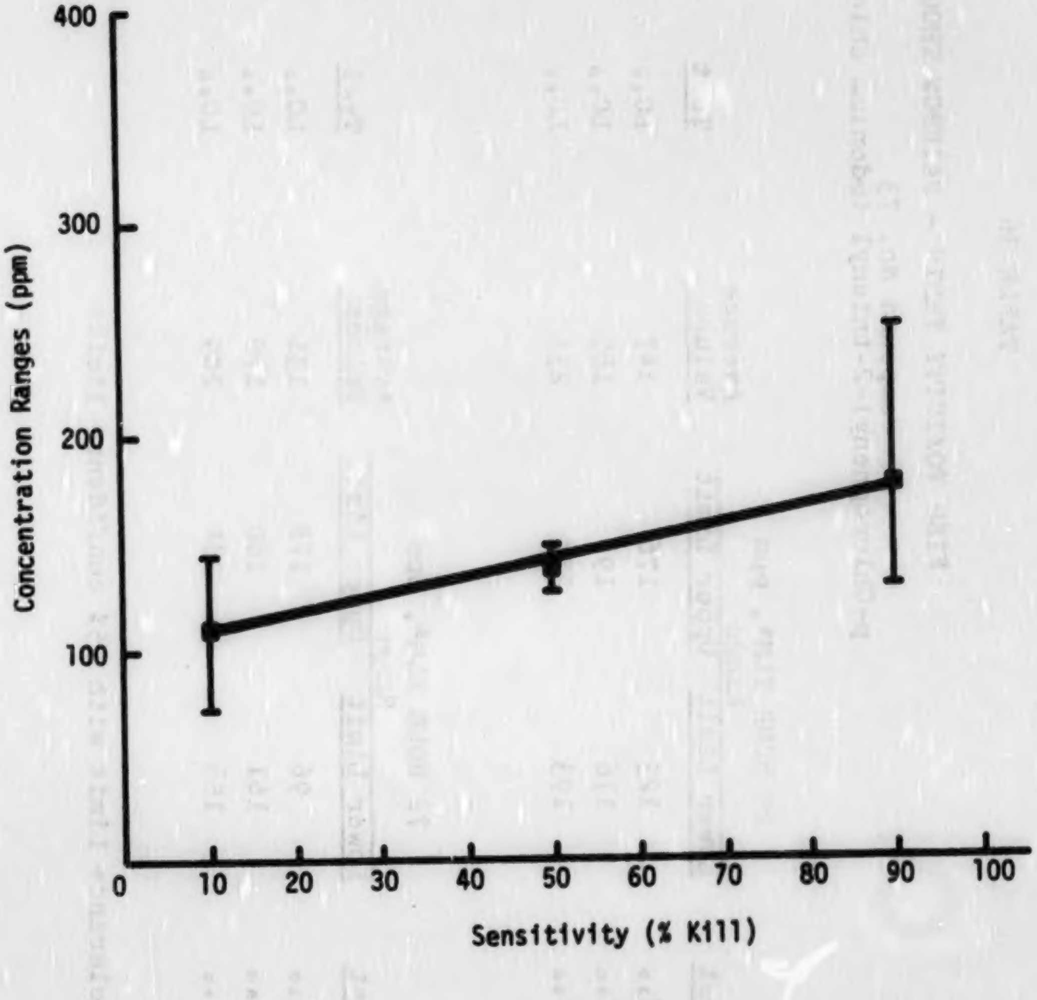


TABLE 36

FISH TOXICITY TESTS - RAINBOW TROUT

Compound No. 73
p-Chlorophenyl-2-thienyl iodonium chloride

24 HOUR TLM*, ppm

Test	Range		Average Values
	Lower Limit	Upper Limit	
LC_{10}	123	176	147
LC_{50}	176	194	185
LC_{90}	193	277	231

48 HOUR TLM*, ppm

Test	Range		Average Values
	Lower Limit	Upper Limit	
LC_{10}	105	178	137
LC_{50}	170	191	180
LC_{90}	182	307	236

72 HOUR TLM*, ppm

Test	Range		Average Values
	Lower Limit	Upper Limit	
LC_{10}	96	178	131
LC_{50}	161	180	170
LC_{90}	154	284	209

96 HOUR TLM*, ppm

Test	Range		Average Values
	Lower Limit	Upper Limit	
LC_{10}	39	245	98
LC_{50}	140	171	155
LC_{90}	98	615	246

*Tolerance Limit with 95% confidence limits

TABLE 37

LABORATORY SCREENING TESTS

Test Compound versus Anabaena flos-aquae
values in ppm

Serial Number	Name of Compound	Activity at Various Concentrations 1.6	0.8	0.4	0.2
74	(p-Bromophenyl)-2-thienyl iodonium chloride	100	95	59	--
78	2-Thienyl-p-tolyliodonium chloride	94	65	48	--
82	(o-Chlorophenyl)-2-thienyl iodonium chloride	95	94	92	--
96	2,5-Dibromo-3,4-dinitrothiophene	100	100	95	--
98	Tetrachlorothiophene	23	23	14	--
121	N'-(3-(1,1-Dimethylethyl)-4-nitrophenyl)-N,N-dimethyl urea	--	9	--	--
124	N-(4-Butyl-2-nitrophenyl)-acetamide	--	0	--	--
126	7,8-Dihydro-6H-pyrrolo(1,2-e) purin-4-01	--	0	--	--
127	2-Tert-butyl-4-nitrophenol	--	12	--	--
136	2,2'-(1,2-Ethenediyl)bisbenzoxazole	100	91	68	41
146	5-Butyl-2-methyl-1H-benzimidazole	--	0	--	--

TABLE 37 continued

Serial Number	Name of Compound	Activity at Various Concentrations 1.6	0.8	0.4	0.2
147	Pentachlorophenol, compound with 2-(2,4,5-Trichlorophenoxy)ethanamine (1:1)	--	0	--	--
148	N'-(4-((6-Chloro-4-trifluoromethyl)-2-pyridinyl)oxy)-N,N-dimethyl urea	--	0	--	--
149	5-Nitro-2-thiophenecarboxaldehyde, oxime	--	0	--	--
150	N-(4-((6-Chloro-2-pyridinyl)oxy)phenyl) acetamide	--	0	--	--
151	N-(4-((6-Chloro-2-pyridinyl)oxy)phenyl)-N'-methyl urea	--	0	--	--
152	N'-(4-((2,6-Dichloro-4-pyridinyl)oxy)phenyl)-N,N-dimethyl urea	--	0	--	--
153	2,5-Bis((4-methylphenyl)sulfonyl)-3,4-dinitrothiophene	--	8	--	--
154	N-(3-Chlorophenyl)-2-isoxazolidinecarboxamide	--	0	--	--
155	N-(4-Chlorophenyl)-2-isoxazolidinecarboxamide	--	18	--	--
156	3-(Acetyloxy)-4-bromobutanoic acid, methyl ester	--	6	--	--
157	N'-(4-Acetylphenyl)-N,N-dimethyl urea	--	0	--	--

TABLE 37 continued

Serial Number	Name of Compound	Activity at Various Concentrations 1.6	0.8	0.4	0.2
158	N,N-Dimethyl-N'-(4-((6-(methylthio)-2-pyridinyl)-oxy)phenyl) urea	--	0	--	--
159	N'-(4-((6-Chloro-2-pyridinyl) oxy)phenyl)-N,N-diethyl urea	--	0	--	--
160	2-(2,4-Dichlorophenoxy)-3-nitropyridine	--	2	--	--
161	Tris(dimethylamino)(hydroxy-phenyl=methyl) phosphonium, hydroxide, inner salt	--	10	--	--
162	N'-(4-((6-Chloro-2-pyrazinyl) oxy)phenyl)-N,N-dimethyl urea	--	0	--	--
163	N'-(4-((3-Chloro-2-pyridinyl) oxy)phenyl)-N,N-dimethyl urea	--	0	--	--
164	N'-(4-Ethylphenyl)-N,N-dimethyl urea	--	0	--	--
165	N'-(4-((3,5-Dichloro-2-pyridinyl)oxy)phenyl)-N,N-dimethyl urea	--	0	--	--
166	4-Amino-6-(1,1-dimethyl=ethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one	--	13	--	--
167	1H-Imidazol-2-ylphenyl-diazene	--	0	--	--
168	2-Chloro-6-(4-methoxy-phenoxy) pyridine	--	0	--	--

TABLE 37 continued

Serial Number	Name of Compound	Activity at Various Concentrations			
		1.6	0.8	0.4	0.2
169	N'-(4-(2,6-dinitro-4-(trifluoromethyl)phenoxy)=phenyl)-N,N-dimethyl urea	--	0	--	--
170	2-(4-Chlorophenyl)-2,3,5,6-tetrahydroimidazo(2,1-b)-thiazole, monohydrochloride	--	14	--	--
171	2,3,5,6-Tetrahydro-2-(2-naphthalenyl)-imidazo(2,1-b), monohydrochloride	--	0	--	--
172	2-Hydroxy-N-phenyl-3-pyridinecarboxamide	--	4	--	--
173	N'-(3-Chlorophenyl-N-methoxy-N-methyl urea	--	0	--	--
176	1,2-Dichloro-4-(isothiocyanato-methoxy) benzene	--	97	--	--
177	1-(2,4,5-Trichlorophenoxy)=thiocyanic acid, ethyl ester	--	65	--	--
178	2-((2-(Dimethylamino)ethyl) amino)3,4-dihydro-1(2H)-isoquinolinone, dihydrochloride	--	0	--	--
179	2-Phenyl-5H-(1,2,4)triazolo-(1,5-b)isoindole	--	0	--	--
180	2-(3-Methylphenyl)-5H-(1,2,4)-triazolo(1,5-b)isoindole	--	0	--	--

Serial Number	Name of Compound	Activity at Various Concentrations 1.6	0.8	0.4	0.2
182	((4,5-Dimethoxy-1,2-phenylene)-bis=(imino(thioxomethylene)))bis-carbamic acid, dimethyl ester	--	0	--	--
183	3-(4-(1,1-dimethyl=ethyl)phenyl)-2,3,5,6-tetrahydroimidazo(2,1-b) thiazole	--	0	--	--
184	Phenyl-2-thienyl methanone, o-((methyl=amino)carbonyl) oxime	--	28	--	--
185	Bis((1,1'-biphenyl)-4-yl)-ethanedione	--	0	--	--
186	((3,5-Dichloro-6-fluoro-2-pyridinyl)oxy)methyl ester thiocyanic acid	--	65	--	--
187	2-(4-(Ethoxyphenyl)2,3,5,6-tetrahydroimidazo(2,1-b)thiazole, monohydrochloride	--	5	--	--
188	N-(1-(4-Bromo-2,5-dichlorophenoxy)-2,2,2-trichloroethyl)formamide	--	0	--	--
189	N'-(4-Ethyl-3-nitrophenyl)-N,N-dimethyl urea	--	3	--	--
190	4-((3,5,6-Trichloro-2-pyridinyl)-oxy)phenol)	--	0	--	--
191	N'-(3-Chloro-4-((6-chloro-2-pyridinyl)oxy)=phenyl)-N,N-dimethyl urea	--	18	--	--
192	N'-(4-((2-Chloro-6-methyl-4-pyrimidinyl)=oxy)phenyl)-N,N-dimethyl urea	--	0	--	--

TABLE 37 continued

Serial Number	Name of Compound	Activity at Various Concentrations			
		1.6	0.8	0.4	0.2
193	N'-(4-((6-Chloro-2-pyridinyl)oxy)-3-(trifluoromethyl)phenyl)-N,N-dimethyl urea	--	41	--	--
194	N,N-Dimethyl-N'-(4-((6-(trifluoromethyl)-2-pyridinyl)thio)phenyl) urea	--	61	--	--
195	N-(1-Methylethyl)-4-phenoxybenzenamine	--	0	--	--
196	N'-(4-((6-Bromo-2-pyridinyl)thio) phenyl)-N,N-dimethyl urea	--	0	--	--
197	1-(3,3-Dichloro-1-methylenebutyl)-3,5-dimethyl benzene	--	20	--	--
198	p-(p-Nitrophenylthio) phenol	--	0	--	--
199	1-((4-Nitrophenyl)methyl)piperidine	--	0	--	--
200	(5-Chloro-2,4,dimethoxyphenyl)-carbamic acid, 2,4,5-trichlorophenyl ester	--	0	--	--
201	2,4-Dibromo-3-methyl-6-nitrophenol	--	0	--	--
202	2,4-Dichloro-3-methyl-6-nitrophenol	--	0	--	--
203	N-Bromophenyl-2-chloroacetamide	--	0	--	--
204	4-((6-Fluoro-2-pyridinyl)=thio) phenol	--	0	--	--

TABLE 37 continued

Serial Number	Name of Compound	Activity at Various Concentrations 1.6	0.8	0.4	0.2
205	1-Chloro-2-(methylsulfonyl)-ethane	--	0	--	--
206	2,4,5-Trichloro-3-methyl-6-nitrophenol	--	0	--	--

TABLE 38

LABORATORY SCREENING TESTS

Test Compounds versus *Microcystis aeruginosa*
values in ppm

Serial Number	Name of Compound	Activity at Various Concentrations			
		1.6	0.8	0.4	0.2
136	2,2'-(1,2-Ethenediyl)bisbenzoxazole	100	94	94	87
192	N'-(4-((2-Chloro-6-methyl-4-pyrimidinyl)=oxy)phenyl)-N,N-dimethyl urea	--	53	--	--
197	1-(3,3-Dichloro-1-methylenebutyl)-3,5-dimethyl benzene	--	64	--	--

Microcystis down to 0.4 ppm. Compound No. 176 exhibited 97 percent control at the 0.8 ppm level when tested against *Anabaena*.

Two commercial algaecides, copper sulphate and Cutrine (copper sulphate with an ethanolamine complexing agent) were tested at a wide range of concentrations against both target species of algae to give a comparative base (Table 39 and Table 40).

Compound Activity as a Function of pH

A test series was run with compounds No. 23 and No. 73 at 0.8 ppm versus *Microcystis aeruginosa* at variable pH values ranging from pH 6 to pH 9. Compound No. 23 showed a decreasing activity at pH values below 9.0. The activity of Compound No. 73 however, exhibited no pH-dependence in the pH ranges and concentrations tested (Table 41).

Correlation of Compound Activity with Biomass

Because of the results of the whole-pond test in which the biomass was very high, a laboratory test was designed to determine the relative effect of increasing algal population densities on the algaecide activity of Compound No. 73. As determined with doubling numbers of *Anabaena* cells, ranging from approximately 0.5×10^6 cells/ml to 9.0×10^6 cells/ml no significant reduction in algaecidal activity was noted, except at the highest algal density used. At this density, 9 to 10 million cells/ml, only 84 percent control was found compared to 100 percent control exhibited by each of the others (Table 42). Up to a relatively dense culture, there appears to be no relationship between culture density and algaecidal effectiveness.

TABLE 39

ALGAECIDAL ACTIVITY OF $CuSO_4 \cdot 5H_2O$ AGAINST TWO SPECIES OF BLUE-GREEN ALGAE

Three-Day Test

	Anabaena flos-aquae			Microcystis aeruginosa		
Concentration ppm	Initial Relative Intensity	Final Relative Intensity	Percent Control	Initial Relative Intensity	Final Relative Intensity	Percent Control
1.0	0.18	0.07	87	0.21	0.02	100
0.8	0.18	0.08	86	0.21	0.04	91
0.6	0.18	0.11	80	0.21	0.05	90
0.4	0.18	0.49	16	0.21	0.15	66
0.2	0.18	0.60	0	0.21	0.41	0
Control No. 1	0.18	0.56	--	0.21	0.44	--
Control No. 2	0.18	0.55	--	0.21	0.43	--

TABLE 40

ALGAECIDAL ACTIVITY OF CUTRINE AGAINST TWO SPECIES OF BLUE-GREEN ALGAE
Three-Day Test

Concentration ppm	Anabaena flos-aquae Initial Relative Intensity	Anabaena flos-aquae Final Relative Intensity	Anabaena flos-aquae Percent Control	Microcystis aeruginosa Initial Relative Intensity	Microcystis aeruginosa Final Relative Intensity	Microcystis aeruginosa Percent Control
4.0	0.18	0.03	95	0.21	0.35	92
3.0	0.18	0.08	85	0.21	0.37	91
2.0	0.18	0.17	70	0.21	0.82	81
1.0	0.18	0.56	0	0.21	0.36	17
0.5	0.18	0.53	0	0.21	0.43	0
Control No. 1	0.18	0.56	--	0.21	0.44	--
Control No. 2	0.18	0.55	--	0.21	0.43	--

TABLE 41

pH SENSITIVITY TESTS ON COMPOUNDS NO. 23 (2,5-DICHLORO-3,4-DINITROTHIOPHENE) AND NO. 73 (P-CHLOROPHENYL-2-THIENYL IODONIUM CHLORIDE) AGAINST *Microcystis*

Test Compound Concentration - 0.8 ppm

Serial Number and pH Value	Initial Relative Intensity	Final Relative Intensity	Percent Control
23 - pH 6	.20	.21	57
pH 6 control	.20	.44	
23 - pH 7	.20	.20	64
pH 7 control	.20	.51	
23 - pH 8	.20	.046	95
pH 8 control	.20	.65	
23 - pH 9	.20	.036	100
pH 9 control	.20	.60	
73 - pH 6	.20	.015	100
pH 6 control	.20	.53	
73 - pH 7	.20	.016	100
pH 7 control	.20	.61	
73 - pH 8	.20	.022	100
pH 8 control	.20	.58	
73 - pH 9	.20	.019	100
pH 9 control	.20	.60	

TABLE 42

ALGAECIDAL ACTIVITY - BIOMASS DEPENDENCE TEST versus *Anabaena flos-aquae* @ 1.6 ppm

Test Vessel	Time & Relative Intensities		Control
	Initial R.I.	Final R.I. 0+4 Days	%
I	2.0	0.56*	84
I-Control	2.0	1.50	--
II	1.0	0.39**	100
II-Control	1.0	0.82	--
III	0.5	0.20**	100
III-Control	0.5	0.55	--
IV	0.25	0.16**	100
IV-Control	0.25	0.48	--
V	0.12	0.11**	100
V-Control	0.12	0.37	--
VI	0.06	0.05**	100
VI-Control	0.06	0.20	--

* Residual R.I. = 0.32
**No living cells microscopically detectable

BIOLOGICAL CONTROL SYSTEMS

Compound Activity Enhancement

During the course of Phase I investigations, a bi-flagellated, yellowish-pigmented algal organism, identified as Ochromonas ovalis, was discovered which was found to ingest cells of the target blue-green algal Microcystis aeruginosa. The phagocytic activity of this organism was found to be enhanced by the presence of certain test compounds at low levels which alone exhibited only marginal algaecidal activity. Through Phase II additional synergistic compounds were identified, and environmental and growth parameters, which exhibited a positive affect on the phagocytic activity of various species of Ochromonas, were delineated.

An investigation of this phenomena with respect to three compounds (Nos. 114, 117 and 119) received for testing during Phase III verified the previously obtained data.

Ochromonas danica in the presence of Compound No. 117 (N-((4-(6-cyano-2-pyridinyl)oxy)phenyl)-N,N-dimethyl urea) and No. 119 (N'-(4-((6-bromo-2-pyridinyl)oxy)phenyl)-N,N-dimethyl urea) both produced 100 percent control of Microcystis in six days, compared to 92 percent control in the same period of time where no test compound was used. Compound No. 114 showed no activity-enhancing effect at the 0.2 ppm level, as did No. 117 and No. 119 (Figure 14, Table 43, and Appendix E). In no case however, was the system improvement considered to be of sufficient magnitude to warrant further investigation with the compounds tested. The phagocytic activity enhancement phenomenon may still be valid, however, should a compound with sufficient optimizing properties be discovered.

TABLE 43

INFLUENCE OF TEST COMPOUND NO. 117 - N((4-(6-cyano-2-pyridinyl)oxy)phenyl-N,N-dimethylurea AT 0.2 PPM ON THE PHAGOCYTIC ACTIVITY OF FOUR SPECIES OF *Ochromonas*

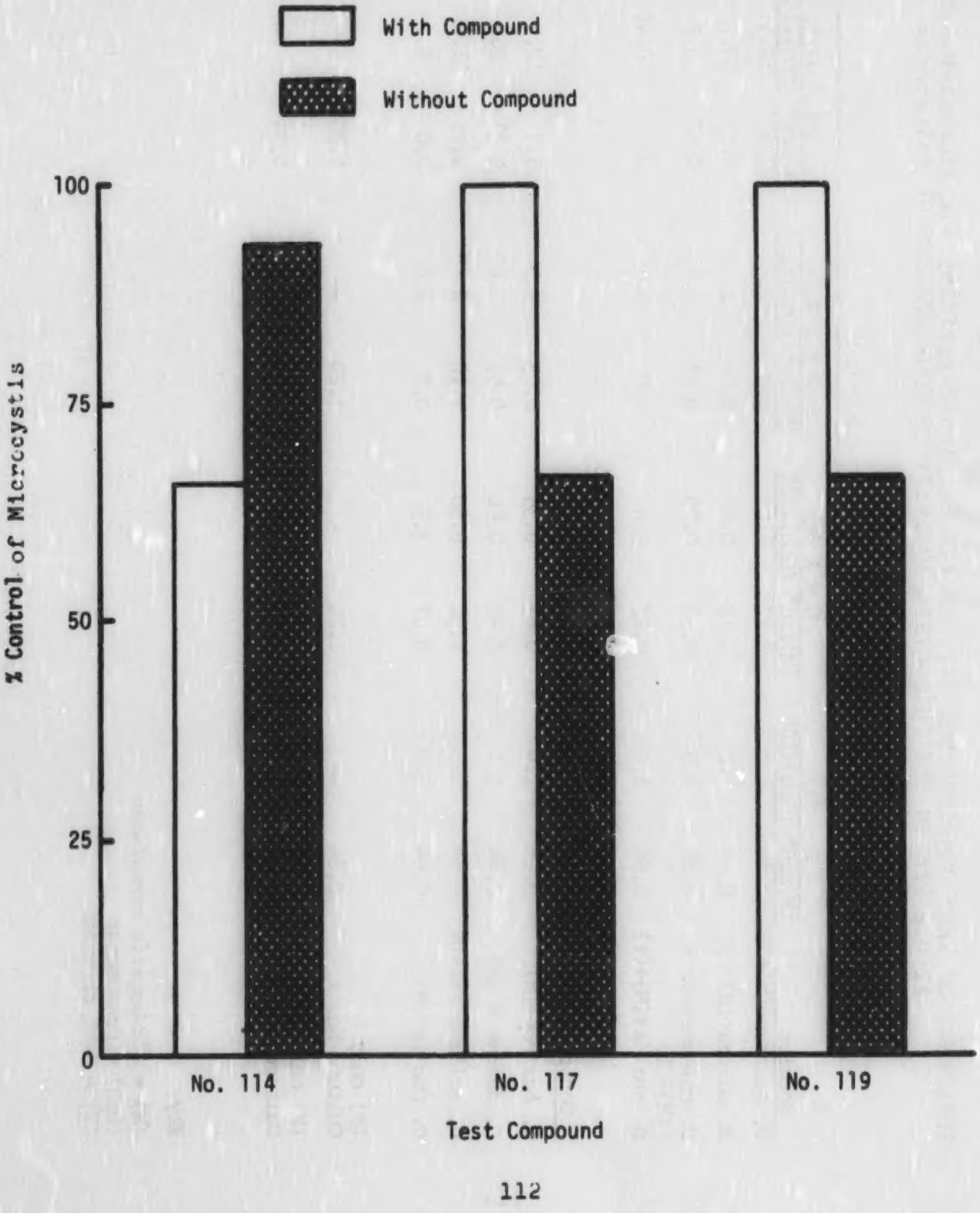
	CELL COUNTS								
	0 - days		0 + 1 day		0 + 2 days		0 + 6 days		
Species	(M)x10^6	(Och)x10^4	(M)x10^6	(Och)x10^4	(M)x10^6	(Och)x10^4	(M)x10^6	(Och)x10^4	Percent Control
O. bastrop+(M)+(T)	0.84	1.0	0.47	1.80	0.0	7.3	0.0	12.9	100
O. danica+(M)+(T)	0.84	1.0	0.73	0.65	0.19	1.9	0.0	13.7	100
O. malhamensis + (M)+(T)	0.84	1.0	0.98	0.30	0.95	0.70	0.57	10.5	86
O. ovalis+(M)+(T)	0.84	1.0	0.07	3.0	0.0	9.1	0.0	0.40	100
Controls									
O. bastrop+(M)	0.84	1.0	0.63	0.35	0.03	4.5	0.0	12.2	100
O. danica + (M)	0.84	1.0	0.42	0.40	0.53	2.60	0.34	2.0	92
O. malhamensis+(M)	0.84	1.0	0.90	0.50	1.10	0.45	0.75	11.2	82
O. Ovalis+(M)	0.84	1.0	0.07	3.5	0.0	8.6	0.0	8.2	100
(M) only Culture No. 1	0.84	—	1.20	—	1.20	—	4.35	—	—
(M) only Culture No. 2	0.84	—	1.16	—	1.06	—	4.25	—	—

Key:

(M) = *Microcystis aeruginosa*
(Och) = *Ochromonas*
(T) = Test chemical

Figure 14.

Influence of Three Test Compounds on the Phagocytic Activity of *Ochromonas danica* at 0.2 ppm



Storage-Viability Study

A comprehensive study, involving 108 separate tests, was undertaken to determine the various conditions under which the Ochromonas ovalis and Ochromonas bastrop species would be able to maintain viability and return to normal activity upon re-culturing. Each species was subjected to seven different types of substrate media, four different moisture states, and three widely different temperature conditions.

After 15 days storage time under the specified conditions, samples from each culture were re-cultured in Gorham's medium for three days and then examined microscopically. Those cultures which showed viable Ochromonas cells still present for 44- and 70-day periods are indicated with an asterisk in Table 44. Both species survived the full 70-day test period in moist activated charcoal, in jellied liquid nutrient agar at room temperature, and when imbibed in cotton or polyester fibers under air-dried conditions.

In no case of survival was the number of reviving cells very great but the fact that cell viability was retained at all furnishes a basis for the possibility of storage of substantial culture volumes especially since the cells were able, in certain substrates, to survive dry or partially dried conditions for such a long period of time.

Field Tests

The planned approach toward further development of the phagocytic organisms, for the natural control of Microcystis blooms was to introduce a culture of Ochromonas ovalis into a Microcystis-infested body of water, or into an isolated portion thereof. This chrysomonad algal species had proven to be a voracious feeder on Microcystis aeruginosa during

TABLE 44

Ochromonas STORAGE-VIABILITY STUDY

Conditions which Produced Positive Results	0+15 days		0+44 days		0+70 days	
	O. ovalis	O. bastrop	O. ovalis	O. bastrop	O. ovalis	O. bastrop
Gorham's medium - refrigerated	**	*				
Gorham's medium - room temperature	*	*				
Gorham's medium - refrigerated, evaporated (near dryness)	**	**				
Gorham's medium - room temperature, evaporated (near dryness)	*	*				
Topsoil - refrigerated, sterilized, moist	*	*	?	?		*
Topsoil - room temperature, sterilized, moist	*	*	?	?	*	
Topsoil - refrigerated, sterilized, dry	*	*				
Topsoil - room temperature, sterilized, dry	*	*				
Topsoil - refrigerated, non-sterilized, moist	*	*				
Activated charcoal - room temperature, moist		*	*	*	*	*
Nutrient agar (gel) - refrigerated	*	**				
Nutrient agar (gel) - room temperature	*	**	*	*	*	*

TABLE 44 continued

Conditions which Produced Positive Results	0+15 days		0+44 days		0+70 days	
	O. ovalis	O. bastrop	O. ovalis	O. bastrop	O. ovalis	O. bastrop
Liquid nutrient agar - refrigerated	*	**	*	*	*	
Liquid nutrient agar - room temperature	**	**	?	?	*	*
Polyester fibers - refrigerated, dried		**				
Polyester fibers - room temperature, dried	*	*	?	?	*	*
Polyester fibers - refrigerated moist	*	*				
Cotton fibers - refrigerated, moist	*	**	?	?		*
Cotton fibers - room temperature, moist	*	*	*	?		*
Cotton fibers - room temperature, dried		*	*	*	*	*

* Viable organisms present after 15 days storage or longer
**Copious number of viable organisms present after 15 days or longer

extensive laboratory and small-scale field test situations. Verification of these results under natural conditions was attempted as a sub-portion of the whole-pond test in North Texas.

Sixty-gallon plastic bags, identical to those used for control chambers, were filled with ambient pond water and covered with plastic previous to administration of test compound to the pond. The dense cultures of Ochromonas were added to each test chamber sufficient to yield an Ochromonas density of about 200 cells/ml. The initial Microcystis population was monitored at 10,000 cells/ml.

At 0+1 day water samples were taken from each vessel and examined microscopically to determine algal population densities. Data from this and subsequent monitorings indicated that the physical, chemical, and/or biological interference conditions were apparently such that the Ochromonas could not cope with them and this algal species did not survive.

Ultramicrostructure and Mode-of-Action Study

An important aspect of the biological control study was undertaken by Drs. Michael J. Wynne and Gary Cole of the University of Texas Botany Department. An extensive investigation on the sub-cellular organelle structure of Ochromonas danica and their mode of ingestive action was made during Phase III. The complete report is contained in Appendix A.

In summary, Wynne and Cole found that the chrysomonad alga (Ochromonas) is a weak autotroph, primarily because of insufficient chlorophyll, and therefore requires other organic materials for sources of nitrogen, and for carbon energy sources. Consequently, the endocytic mode of nutrition is common with this organism, of which the toxic blue-green alga *Microcystis aeruginosa* is a common target when present in the same culture.

A study of the efficiency and mechanism by which this phagocytic organism engulfs and ingests cells of *Microcystis* utilizing various light and electron-microscopic techniques, was the primary focus of this investigation. The action of *Ochromonas* on *Microcystis* is suggestive of a mechanism for this nuisance algal species.

Dense cultures of *Ochromonas danica* were prepared by the University investigators and added to existing healthy stock cultures of *Microcystis*. The decline in *Microcystis* cell count as a function of time is shown graphically in Table 1, Appendix A. The rate of endocystis is seen to be very rapid during the initial 10 to 15 minute contact period, after which the action declines markedly.

A series of light micrographs taken at successive time intervals show various stages of engulfment of *Microcystis* by *O. danica* and subsequent intercellular digestion of the former (Figures 1-4, Appendix A). Ingestion has been observed to consistently occur at the anterior end of the phagocyte. When *Microcystis* is initially engulfed it becomes invaginated in the primary food vacuole and then immediately begins to migrate toward the posterior pole of the cell. This food vacuole soon fuses with the larger secondary food vacuole and releases its contents into the digestive organelle. These events may occur several successive times, resulting in the accumulation of as many as six to eight *Microcystis* cells in the secondary phagosome, which reaches its maximum volume when the vacuole becomes distended to full capacity, at which time O. danica ceases any further engulfment.

The precise extent of digestive activities in this stage is unknown, but it is suggested that at least the outer, mucilaginous sheath of the blue-green alga is removed.

A unique cross-fracturing freeze-etch method with scanning electron micrography techniques was employed by Drs. Wynne and Cole in further investigations of the digestive processes within the secondary phagosome. Almost complete breakdown of the engulfed cells is indicated in the secondary phagosome (SP) in Figure 4, Appendix A.

Figures 11 through 15, Appendix A, show Microcystis in successively later stages of digestion, in which the photosynthetic lamellae are seen to gradually depart from their recognizable pattern to one of discontinuity. The ends of these membranes apparently tend to fuse and give the appearance of circular vessicles.

A freeze-etched, cross-fractured golgi complex, or dictyosome, is shown just above the nucleus in Figure 21, Appendix A. This organelle, which is responsible for the production of digestive enzymes, was in an active state of proliferation at the time of fixation, and treatment with acid phosphates resulted in the accumulation of electron-rich deposits concentrated in the vessicles arising from the golgi cisternae, as seen in Figure 22.

A number of other kinds of microorganisms, including brewer's yeast and several species of bacteria, have also been observed to be ingested within the food vacuoles of O. danica. Figure

25, Appendix A, shows seven partially digested bacteria enclosed with a secondary phagosome.

Observations of endocytotic activity under experimental conditions seem to indicate that initiation of the action depends upon a chance contact between the two cells. Since invagination seems to always occur at the anterior end of the chrysomonad, its beating flagella tend to create microcurrents over the surface of the cell, thus causing any posterior contacts to be moved in that direction. Aaronson postulated that the flagella may be involved in the accretion of particles on the cell surface and thus provide the organsim with a mechanism of sampling material from the environment.

Since *Microcystis* *aeruginosa* is a common bloom-producing alga and one which is capable of releasing toxic substances, identified as cyclic polypeptides, in sufficiently high concentrations to kill a variety of animals the concept of developing a natural control mechanism which could be introduced at an appropriate time, is intriguing.

In this study, the rate of endocytosis was determined by observing the rate of decrease in concentration of *Microcystis* in a culture which contained a known concentration of *O. danica*. The uptake of *Microcystis* was most rapid during the first ten minutes after the two cultures were combined, at a calcualted 7.0×10^4 cells/ml/min. The rate of invagination then decreased to 1.0×10^4 cells/ml/min during the next 20 minutes primarily due to two factors: reduction of blue-green cell concentration, thus reducing the number of change-encounters with *Ochromonas* cells, and many of the *Ochromonas* cells becoming gorged with blue-green algae and thus rendering themselves incapable of additional feeding until the ingested cells had decomposed.

It is suggested that the effectiveness of utilizing such a phagocytic organism in the biological control of Microcystis blooms would depend on fulfillment of at least the following four conditions; a continuing rapid rate of intercellular digestion of Microcystis, the maintenance of high concentrations of Ochromonas in the environmental niche, the selective action on Microcystis by Ochromonas as a preferential food source and the detoxification of Microcystis cells during the digestive process, all of which will require further investigation.

SECTION VI

REFERENCES

Aaronson, S. Particle Aggregation and Photoautotrophy by Ochromonas. Arch. Mikrobiol. 29:39-44, 1973.

Bain, R. C., Jr. Algal Growth Assessments by Fluorescence Techniques. Proceedings of the Eutrophication Biostimulation Assessment Workshop, U. S. Department of the Interior, Federal Water Pollution Control Administration, 1969.

Bartsch, A. F. Practical Methods for Control of Algae and Water Weeds. Public Health Rpt. 69:749-757, 1954.

Bartsch, A. F. Proceedings of a Symposium Jointly Sponsored by University of Washington and Federal Water Pollution Control Administration. U. S. Department of the Interior, 1967.

Battelle, Columbus Laboratories. Effects of Chemicals on Aquatic Life. In: Water Quality Data Book, Vol. 3. U. S. Environmental Protection Agency, 1971.

Brown, R. L. Effects of Light and Temperature on Algal Growth. Calif. Dept. of Water Res, 1973.

Bueltman, C. G. Provisional Algal Assay Procedure. Joint Industry-Government Task Force on Eutrophication, 1969.

Cortell, J. M. The Role of Herbicides in the Preservation of our Urban and Industrial Water Resources. Weeds, Trees, and Turf, 6:12-28, 1970.

Drews, G. Fine Structure and Chemical Composition of the Cell Envelopes. In: The Biology of Blue-Green Algae, Botanical Monographs, Vol. 9, Carr, N. G. and Whitton, B. A. (eds.). Berkeley, Univ. of Calif. Press, 1973. pp. 99-116.

Environmental Protection Agency. Eutrophication Research Highlights. Pacific Northwest Environmental Research Laboratory, September 1973.

Faust, S. D. Fate of Organic Pesticides in the Aquatic Environment Am. Chem. Soc., Washington, DC, 1972.

Fitzgerland, G. P. Algaecides. Univ. of Wis., Madison, 1971.

Goddard, E. D. Molecular Association in Biological and Related Systems. Am. Chem. Soc., Washington, DC, 1968.

Gorham, P. R. Toxic Algae as a Public Health Hazard. JAWWA. 56:1481-8, 1964.

Hasler, A. D. Antibiotic Aspects of Copper Treatment of Lakes. Wis. Acad. Sci., Arts and Lett. 39:97-103, 1947.

Holm-Hansen, O., et al. Fluorometric Determination of Chlorophyll. Inst. of Marine Resources, Scripps Institute of Oceanography, 1966.

Highes, E. O., P. R. Gorham and A. Zehnder. Toxicity of a Unialgal Culture of Microcystis aeruginosa. Canadian J. of Microbiol. 4:225-236, 1958.

Hutchinson, G. E. A Treatise on Limnology. Vols. I and II, John Wiley & Sons, Inc., New York, NY, 1967.

Jackson, Daniel F. Algae, Man and the Environment. Syracuse University Press, Syracuse, NY, 1967.

Katz, M. and A. R. Gaufin. The Effects of Pollution on the Fish Population of a Midwestern Stream. Trans. Am. Fish Soc. 82:156-165, 1953.

Keup, L. E., W. M. Ingram, and K. M. MacKenthun. Biology of Water Pollution. U. S. Department of the Inteiror, Federal Water Pollution Control Administration, June 1968.

Kuentzel, L. E. Bacteria, Carbon Dioxide, and Algal Blooms. JWPCF. 41:10,1737-1747, 1969.

Lewin, R. A. Physiology and Biochemistry of Algae. Academic Press, New York, NY, 1962.

MacKenthun, K. M. and W. M. Ingram. Limnological Aspects of Recreational Lakes. U. S. Dept. of Health, Ed. and Welfare, 1964.

MacKenthun, K. M. and W. M. Ingram. Biological Associated Problems in Fresh Water Environments - Their Identification, Investigation, and Control. U. S. Dept. of the Interior, Fed. Water Poll. Control Adm., 1967.

MacKenthun, K. M. and C. D. McNabb. Stabilization Pond Studies in Wisconsin. JWPCF 33(12):1234-1251, 1961.

Malueg, K. W., et al. Lake Restoration by Nutrient Removal from Waste Water Effluent. National Environmental Res. Center, Office of Res. and Monitoring, 1973.

Meyer, J. H. Aquatic Herbicides and Algaecides. Noyes Data Corporation, 1971.

Middlebrooks, E. J., T. E. Maloney, E. F. Powers, and L. M. Knack. Proceedings of the Eutrophication-Biostimulation Assessment Workshop. Sanitary Eng. Res. Lab, Univ. of Calif. and U.S. Dept. of the Interior, Fed. Water Pol. Control Adm., 1969.

Moyle, J. B. The Use of Copper Sulphate for Algae Control and its Biological Implications. Am. Assoc. for the Advancement of Science, Washington, DC, 1949.

Nichols, M. S., T. Henkel, and D. McNall. Copper in Lake Muds from Lakes of the Madison Area. Trans. Wis. Acad. Sci., Arts and Lett. 38:333-350, 1946.

Okino, T. Studies on the Blooming of *Microcystis aeruginosa* Jap. J. Bot. 20:381-402, 1973.

Otto, N. E. and T. R. Bartley. Aquatic Weed Control Studies. U. S. Dept. of the Interior, Bureau of Rec., Res. Rpt. No. 2, 1966.

Palmer, C. M. Algae in Water Supplies. U. S. Dept. of Health, Ed., and Welfare, Washington, DC, 1962.

Prescott, G. W. How to Know the Fresh Water Algae. Wm. C. Brown Publishing Company, 1970.

Prows, B. L. Development of a Selective Algaecide to Control Nuisance Algal Growth. U. S. Dept. of the Interior, Washington, DC, 1971.

Prows, B. L. and W. F. McIlhenny. Development of a Selective Algaecide to Control Nuisance Algal Growth - Phase II. U. S. Environmental Protection Agency, 1973.

Reazin, G. H., Jr. On the Dark Metabolism of Golden Brown Algae, *Ochromonas malhamensis*. Am. J. Bot. 41:9, 771-777, 1954.

Reazin, G. H., Jr. The Metabolism of Glucose by the Alga Ochromonas malhamensis. Plant Phys. 31:4, 229-303, 1956.

Smith, G. M. The Fresh Water Algae of the United States. McGraw-Hill Book Company, New York, NY, 1950.

U. S. Environmental Protection Agency. Water Quality Data Book, Vol. 3. Effects of Chemicals on Aquatic Life, 1971.

Vance, B. D. Sensitivity of Microcystis aeruginosa and Other Blue-Green Algae and Associated Bacteria to Selected Antibiotics. J. of Phycology. 2:125-128, 1966.

Weiss, C. M. The Relative Significance of Phosphorus and Nitrogen as Algal Nutrients. Univ. of N. Carolina, Chapel Hill, NC, 1970.

Zajic, J. E. Properties and Products of Algae. Proceedings of the Symposium on the Culture of Algae sponsored by the Am. Chem. Soc., Plenum Press, NY, 1970.

SECTION VII

APPENDICES

APPENDIX A

ENDOCYTOSIS OF *MICROCYSTIS AERUGINOSA* BY *OCHROMONAS DANICA*

by

Garry T. Cole
and
Michael J. Wynne

Department of Botany, University of Texas

Austin, Texas 78712

ABSTRACT

Ochromonas danica, a chrysomonad alga which demonstrates a high degree of nutritional versatility, is capable of feeding on the toxic blue-green alga, Microcystis aeruginosa Kutz. In this paper, light-microscopic, electron-microscopic, and cytochemical examinations of endocytosis in O. danica are reported with particular emphasis on the vicissitudes of phagosomal and lysosomal activities during intracellular digestion. A diagrammatic interpretation of the function of organelles associated with endocytosis is presented.

INTRODUCTION

Ochromonas danica Prings. demonstrates an unusual versatility of nutritional modes. This chrysomonad alga is a weak autotroph, primarily because of insufficient chlorophyll, and therefore, requires some organic substances in the media to serve both as a source of nitrogen compounds and as a carbon and energy source. Pringsheim (43) suggested that limited photosynthesis helps to keep Ochromonas alive during long periods in axenic culture but, in the absence of an added carbon source, photosynthesis is insufficient to provide all the organic compounds necessary for continued multiplication. However, Aaronson and Baker (3) have reported that O. danica can grow photoautotrophically in a substrate-free medium in 5 percent CO_2 on a shaker and illuminated with fluorescent lamps. If particles of organic material are added to axenic cultures of O. danica, or, if the cultures become contaminated with other microorganisms such as bacteria, this chrysomonad also demonstrates adeptness in endocytotic activity. Pringsheim (43) considered the nutritional versatility of Ochromonas to be a primitive character, perhaps reflecting the mixed trophic levels of ancestral forms. It is the endocytotic mode of nutrition which will be explored in this paper. The efficiency by which the chrysomonad engulfs and ingests Microcystis aeruginosa Kutz., a toxic blue-green alga, is suggestive of a mechanism of biological control and was examined using light-microscopic and electron-microscopic techniques.

MATERIALS AND METHODS

Pure cultures of *Ochromonas danica* and *Microcystis aeruginosa* were obtained from the Indiana Culture Collection (L1298) and Dow Chemical Company, Freeport, Texas, respectively. A culture of *M. aeruginosa* was also obtained from Dr. Franklyn Ott (#0510). The culture techniques as well as the thin-section and freeze-etch preparatory procedures for these organisms were outlined in an earlier paper (12). Light-microscopic studies of endocytosis were performed using a Zeiss universal microscope equipped with Nomarski objectives and a high-intensity light source. Kodak Plus-X film was used.

For estimates of the rate of endocytosis, the following procedure was designed. Axenic cultures of *O. danica* and *M. aeruginosa* were first grown as outlined above, except that equal volumes of inocula of the former were added to 200 ml of *Ochromonas* media in 500 ml Erlenmyer flasks and subjected to continuous agitation (2.2 cps) in a Gyrotory shaker (New Brunswick Sci. Co.). After four days, cell counts of *O. danica* were estimated using a Spencer (No. 1492) hemacytometer. Concentrations of 2.8×10^6 - 3.5×10^6 cells/ml were recorded. Similarly, cell counts were estimated for *M. aeruginosa* after three weeks' growth in stationary culture. Concentrations of about 1.9×10^6 cells per ml were routinely recorded. Clumping of *M. aeruginosa* cells affected both our estimates of cell concentration in axenic culture and our determination of the rates of endocytosis in the mixed condition. The latter problem arose from the fact that *O. danica* was unable to engulf large clumps of cells, but the chrysomonads would cluster in large numbers about the mass of blue-green algae and thus significantly affect the validity of the hemacytometer values. However, we found that vigorous shaking of the test tube which contained the M. aeruginosa culture for approximately five minutes on a Vortex mixer before combining it with O. danica considerably reduced this problem. Aliquots of O. danica and M. aeruginosa at known cellular concentration were then mixed. One-ml samples were subsequently removed from the mixed cultures of three-minute intervals, immediately fixed with one drop of 1 percent formalin, and the number of remaining M. aeruginosa cells was recorded using the hemacytometer. These successive recordings of blue-green algal cell counts were continued for a total period of 30 minutes for each mixed culture examined.

The electron-microscopic, cytochemical procedures for locating acid phosphatase activity (25) in O. danica was based on the technique reported by Stoltze et al.(51), except that our material was incubated at 37°C for 60 minutes. The reaction was monitored under the light microscope by withdrawing cell samples from the Gomori media at 15-minute intervals, centrifuging, and then resuspending the cells in ammonium sulfide. The cells were mounted on microscope slides and examined for the degree of precipitation. Our control experiment included incubation at pH 5.0 without the β-glycerophosphate substrate. Observations of sectioned material before and after uranyl acetate and lead citrate staining indicated that no artifacts were introduced by the post-staining procedures.

OBSERVATIONS

A sequence of light micrographs illustrates different stages of engulfment of M. aeruginosa by O. danica (Figures 1 and 2) and subsequent intracellular digestion of the former (Figures 3 and 4). In Figure 1, contact between a blue-green alga (arrow) and O. danica has occurred at the anterior end of the chrysomonad, adjacent to its contractile vacuole (cv). It is in this region of *O. danica* that engulfment consistently occurred. In the same chrysomonad cell in Figure 1, another blue-green alga (arrowhead) is enclosed by a vacuole which has produced a distinct bulge on the lateral surface of the phagocyte. A lateral protrusion of the cell membrane encasing a *Microcystis* cell is even more evident in Figure 2. At the posterior end of *O. danica* in Figure 2, a *Microcystis* cell is contained by another vacuole. It is suggested that two major kinds of vacuoles form during endocytosis in *O. danica*. When *M. aeruginosa* is initially engulfed by the chrysomonad, the prokaryote is enclosed by a primary food vacuole, or, primary phagosome (pp), which immediately begins to migrate toward the posterior pole of the cell. It is this organelle and its contents which cause the lateral distention of the phagocyte. The primary phagosome soon fuses with the larger secondary food vacuole, or secondary phagosome (sp, Figures 2, 3, and 4), and releases its contents into this digestive organelle. The preceeding successive events can occur several times resulting in the accumulation of six to eight *Microcystis* cells in the secondary phagosome (Figure 3). Soon after engulfment of the first blue-green alga, *O. danica* cells lose their characteristic pyriform shape and become almost spherical. The cell reaches its maximum volume when the secondary phagosome becomes distended to its full capacity as indicated in Figure 3. At this stage, *O. danica* ceases further engulfment of *M. aeruginosa*, and the blue-green algal cells contained by the secondary phagosome appear to be digested simultaneously. The precise extent of digestive activities within the primary phagosome is unknown, but we suggest that at least the outer, mucilaginous sheath of the blue-green alga (17) is removed. Digestion within the secondary phagosome has been demonstrated by the transmission electron-microscopic and freeze-etch micrographs which follow. Almost complete breakdown of *M. aeruginosa* cells is indicated in the secondary phagosome in Figure 4.

The rate of endocytosis was based on the rate of decrease of *M. aeruginosa* when mixed with a culture of *O. danica*. As outlined above, known volumes and concentrations of constantly agitated cultures of *O. danica* were combined with an equal volume of known cell concentration of *M. aeruginosa*. The mean concentration of *O. danica* was 3.1 x 10^6 cells/ml and the average concentration of *M. aeruginosa* in axenic culture was 1.9 x 10^6 cells/ml. The results of the hemacytometer counts of free *M. aeruginosa* cells in the presence of *O. danica*, after varying periods, were compiled and assimilated in a graph which is presented in Table I. The greatest rate of decrease of *M. aeruginosa* (i.e., rate of endocytosis) occurred within the first 10 minutes after mixing the two cultures. The actual decrease in the concentration of blue-green algal cells during this initial period, as indicated in Table I, was 7.0 x 10^5 cells/ml. In contrast, the decrease in concentration of *M. aeruginosa* during the subsequent 20 minute period was only 1.0 x 10^5 cells/ml.

Figure 5 shows a freeze-etched, cross-fractured secondary phagosome of *O. danica* which contains the partially digested remains of *Microcystis* and *Ochromonas* cells. The latter demonstrates the cannibalistic nature of the chrysomonad even in the presence of adequate food material. However, the degree of cannibalization when cultures of *O. danica* and *M. aeruginosa* were mixed was minimal and was, therefore, not taken into consideration when preparing Table I. Upon comparison of the blue-green alga enclosed by the secondary phagosome in Figure 5 with the free-living, freeze-etched cell in Figure 6, it is evident that the outer wall of the ingested *M. aeruginosa* has been digested, most of its vacuoles have disappeared, and its photosynthetic lamellae are hypertrophied and irregularly arranged. Crystalloids are frequently present in the cytoplasm of M. aeruginosa (Figures 6 and 7). The periodicity of the lattice pattern of the cross-fractured structure is demonstrated in Figure 6. These inclusions resemble the cytoplasmic structures identified earlier as polyhedral bodies (37, 42). The crystalloids are electron dense in blue-green algal cells which are present in secondary phagosomes of O. danica (Figure 11) and appear to decompose rather slowly (Figure 14).

The thin-sectioned phagocyte in Figure 8 shows M. aeruginosa enclosed within a secondary phagosome. The posterior portion of the chrysomonad has collapsed slightly as a result of the fixation procedure, but the phagosomal membrane and plasmalemma can still be distinguished. This section demonstrates many of the characteristic ultrastructural features of O. danica. The single, anteriorly located dictysome (D) lies adjacent to the contractile vacuole. The large nucleus (N) is located between the lobes of the chloroplast, and the outer membrane of the nuclear envelope is continuous with a sac encompassing the chloroplast (arrows), referred to as the chloroplast ER (9, 23, 24, 50). A large number of mitochondria are distributed around the perimeter of the cell. Also noteworthy is the sparsity of endoplasmic reticulum which is characteristic of O. danica.

In the posterior region of the cell in Figure 8, above the secondary phagosome, a distinct clusture of vesicles is present. Most of these organelles, which are shown at higher magnification in Figure 9, contain membrane fragments, diffuse material, and small vesicles. These contents seem to be common to all vesicles of similar size which are located adjacent to the secondary phagosome (Figures 11 through 14, and 16 through 18). In addition, another kind of vesicle is present in this region of the cell, which is coated with pentagonal and hexagonal subunits (arrow in Figure 9 and 10). These subunits may entirely encase the membrane of the vesicle (Figure 10), or, only partially coat its outer surface (Figures 13 and 18). Similar profiles were illustrated in an earlier ultrastructural examination of _O. danica_ in which the subunits formed the coating of certain vesicles arising from the Golgi complex (12). Suggestions of the function of these structures in association with the process of endocytosis are presented in the discussion.

The blue-green algal cell in Figure 8 lacks its outer, mucilaginous sheath, but the wall is still intact which suggests that the cell had moved from the primary to secondary phagosome just prior to fixation. Of course, it is quite probable that the secondary phagosome in Figure 8 also contained other _M. aeruginosa_ cells. This is also true for the contents of the secondary phagosome shown in Figure 11. In this case, early signs of cytoplasmic decomposition of the blue-green alga are demonstrated. Myelin figures, which have been attributed to pathological alterations of cells (28,46) and cytoplasmic degeneration (13) in a number of different organisms, are found associated with the breakdown products of blue-green algae at early (Figure 11) and late stages of digestion (Figure 14). Electron transparent regions in the cytoplasm of the ingested cell in Figure 11 are also visible. Outside the _Microcystis_ cell, but within the lumen of the secondary phagosome in Figure 11, are clusters of ribosomes which are distributed about the perimeter of the digestive organelle. Appressed to the outer surface of the phagosomal membrane are numerous ER lamellae. Endoplasmic reticulum was frequently observed associated with the outer circumference of secondary phagosomes at various stages of the digestive process (Figures 14, 16 and 17) even though it was sparse in other parts of the cell. The phagosomal membrane in Figure 11 is highly convoluted. As Microcystis is further decomposed, the degree of convolution of this membrane decreases (c.f. Figures 11, 14, 16 and 17).

Of particular interest in Figure 11 is the partially coated vesicle (arrow) lying adjacent to the phagosomal membrane. The contents of this vesicle are typical of those uncoated vesicles of similar dimension found next to the secondary phagosome (Figures 9 and 13). At higher magnification (Figure 12) the coated surface is demonstrated to be composed of subunits similar to those illustrated in Figure 10. Uncoated vesicles in Figure 13 are juxtaposed to tangentially sectioned evaginations of the phagosomal membrane, which is suggestive of an origin of these vesicles through a process of blebbing of the secondary phagosome. In addition, numerous smaller vesicles containing diffuse material are found adjacent to both the surfaces of the secondary phagosome and large vesicles and are distributed randomly through the cytoplasm in this region of the cell (arrows in Figures 13 and 18).

The outer wall of M. aeruginosa in Figure 13 has decomposed and, even though the photosynthetic lamellae are still oriented in a recognizable pattern, they are discontinuous. Only remnants of photosynthetic lamellae can be found in Figure 14. Fragments of membrane have accumulated within the phagosome and appear to be emerging from the partially decomposed blue-green algal cell. This phenomenon is more apparent in Figure 15. Some of the membranous fragments (arrows in Figure 15) are continuous with membranes of the almost completely digested M. aeruginosa cell. A tendency for these fragments to form loops within the secondary phagosome is demonstrated by Figures 14 through 16. Apparently the ends of these membranes may subsequently fuse and, in transverse section, therefore, would give the appearance of vesicles (arrowheads in Figure 15).

Figures 16 and 17 illustrate secondary phagosomes which contain the remains of blue-green algal cells in progessively later stages of digestion. Figure 16 demonstrates a proliferation of the amount of membrane loops and fragments within the digestive vacuole, while both phagosomes show a marked decrease in the amount of electron dense material compared to earlier stages of Microcystis decomposition (c.f. Figures 11 and 14). All that remains of the M. aeruginosa cells in Figure 17 are short, linear membrane fragments and some diffuse material. In the cytoplasm of O. danica in Figure 17 electron-dense deposits have accumulated which are interpreted to be lipid droplets. Also in this region of the same chrysomonad, large, distinctive vesicles (rb) are present which do not appear to be structurally connected to the secondary phagosome and their contents consist of only electron-dense deposits and some diffuse material.

Posterior to the secondary phagosome in Figure 18, two vesicles with partially coated surfaces (c.f. Figure 12) are visible. Both vesicles lie adjacent to the cell membrane, which at several locations in this section, is associated with diffuse and rather fibrous material. At one region (ccm) the inner surface of the plasmalemma is coated with subunits similar to those present on the surface of vesicles. The outer surface of the freeze-etched cell membrane of O. danica in Figure 19 (arrows) demonstrates evaginations which are suggestive of exocytotic activity on the cell surface. The freeze-etch micrograph of O. danica in Figure 20 reveals numerous irregularities on the surface fracture of the plasmalemma and membrane surrounding the large secondary phagosome (arrows). Around the cytoplasmic boundary of the phagosome, many small vesicles appear closely appressed to the membrane surface. As previously indicated in thin sections, phagosomal membrane blebbing and fusion are suspected to occur along this interface (Figures 11 and 13). It is suggested that a relationship may exist between these activities and at least some of the circular surface patterns on the phagosomal membrane demonstrated in this freeze-etch micrograph. Similarly, some irregularities of the surface of the cell membrane may be indicative of exocytotic activity.

At the apex of O. danica in Figure 21, a freeze-etched, cross-fractured dictyosome is shown juxtaposed to the anterior surface of the nucleus. The Golgi complex was in an actively proliferating state at the time of fixation. When endocytotic cells were treated with acid phosphatase accumulations of electron-dense deposits were concentrated in vesicles arising from Golgi cisternae (Figure 22). In the secondary phagosome shown in Figure 23, similar electron-dense deposits resulting from the acid phosphatase reaction were dispersed over the surface and throughout the decomposing M. aeruginosa cell. Localized acid phosphatase positive reactions were also shown in large vesicles surrounding the secondary phagosome (Figures 23 and 24).

Ochromonas danica has a voracious appetite for many different kinds of microorganisms including several species of bacteria and brewer's yeast (1). Figure 25 shows at least seven partially digested bacteria enclosed by a secondary phagosome. Current studies of endocytosis of conidia of Oidiodendron truncatum (Robak) Barron, an imperfect fungus, by O. danica will form the basis of a future communication.

DISCUSSION

The initiation of endocytosis of Microcystis aeruginosa by Ochromonas danica seems to depend on chance contact between the two cells, at least under our experimental conditions. If a Microcystis cell comes into contact with the posterior end of the chrysomonad, the beating flagella of the latter create a microcurrent over the surface of the cell which causes the blue-green alga to be carried toward its anterior end. It is in this region, just posterior to the flagella, where engulfment occurs. Aaronson (2) has also observed that flagellar movements caused India ink particles added to the medium to flow over its sides and toward the anterior end of the organism. He has suggested that the flagella may be involved in accretion of particles on the cell surface of O. danica and thus provide the organism with a mechanism of sampling material from its environment.

Microcystis aeruginosa is a well known bloom-producing blue-green alga (41) which is capable of releasing toxic compounds (7, 26, 33), identified as cyclic polypeptides, into its aquatic environment in sufficiently high concentration to kill a variety of animals, except waterfowl (27). The possibility occurred to us that the voracious appetite which O. danica demonstrates for this blue-green alga could be utilized in developing a biological control system. Examination of endocytosis with the light microscope demonstrated that a large number of Microcystis cells could be rapidly engulfed by a single Ochromonas cell. Under defined experimental conditions it was possible to estimate the rate of endocytosis. Jacques (30) suggested that the rate of endocytosis of an organism could be defined in two different ways. In one case, it could be the rate of invagination of the cell membrane during engulfment estimated on the basis of the number of phagosomes of an average diameter formed by the phagocyte per unit time. In the second case, the rate of endocytosis could be extrapolated from the rate of change in concentration of a defined substance in the medium which is being engulged by the phagocyte. Our estimate is based on the rate of decrease in concentration of *M. aeruginosa* in a culture which contains a known concentration of *O. danica*. Uptake of the blue-green alga was most rapid during the first 10 minutes after mixing the two cultures. The actual rate of decrease of *M. aeruginosa* during this interval (Table I) was 7.0 x 10^4 cells/ml/min. On the other hand, sampling mixed cultures during the last 20 minutes of the experimental period indicated that the rate of decrease of *M. aeruginosa* was only 1.0 x 10^4 cells/ml/min. One explanation of this reduction in the rate of uptake demonstrated in Table I is that the occurrence of endocytosis, at least in our artificial culture conditions, is dependent on chance contact between *M. aeruginosa* and its predator. As the concentration of the former drops because of endocytosis, there is a proportional decrease in the number of chance encounters with *O. danica*. Light-microscopic studies of living cells have indicated that cannibalism in *O. danica* is relatively rare in these mixed cultures and is, therefore, ignored in our statistical analyses. However, the rate of decrease of *Microcystis* cells is not linear (Table I) because many *O. danica* cells soon become gorged with blue-green algae (Figure 3) and are then incapable of additional feeding until the ingested cells have decomposed. Therefore, an initial rapid uptake of cells (i.e., first 10 minutes in Table I) is followed by a digestive period. Fewer *O. danica* cells function as phagocytes during the later part of the experimental period, thus further reducing the number of effective encounters with M. aeruginosa cells. These factors would collectively contribute to a marked reduction in the rate of endocytosis in the mixed cultures after an initial period of very rapid cell uptake. The effectiveness of O. danica in biological control of this blue-green alga would depend on fulfillment of at least the following conditions: 1) rapid rate of intracellular digestion of M. aeruginosa; 2) maintenance of high concentrations of O. danica in the environmental niche; 3) selection of M. aeruginosa by O. danica as the preferential food source; and 4) detoxication of M. aeruginosa during the digestive process. All these factors require investigation.

The mechanism of engulfment of Microcystis cells involves formation of a primary phagosome by O. danica. This structure first appears at the anterior end of the chrysomonad when the blue-green alga contacts the cell membrane of the phagocyte in this region. Schuster et al.(49) suggested that the anterior region of a feeding O. danica cell consists of a "cytoplasmic tongue" differentiated by fibers arising from the kinetosomes and pinocytotic activity on the cell surface. A minimal amount of digestion likely occurs within the primary phagosome, because it is only a transitory structure which soon after formation, about 30 to 60 seconds later, fuses with the secondary phagosome located at the posterior end of O. danica. However, it is suggested that the mucilaginous sheath of M. aeruginosa is at least partially removed in the primary phagosomes, which of course assumed the presence of intraphagosomal hydrolase enzymes. Aaronson (1) has presented a lengthy list of hydrolases which have been found in O. danica. There is also evidence that this chrysomonad is capable of secreting a wide variety of hydrolases into its environment (36). The effects of these secreted proteins on the food material which is ingested is unknown.

The large posterior vacuole of O. danica has been referred to as the 'leucosin vacuole' in earlier studies of both axenic cultures (43) and cultures which are feeding on other organisms (15). We prefer to associate the term leucosin with the large, posterior vacuole and storage product which occur in axenic cultures of the chrysomonad grown under conditions of adequate light and carbon supplement. In this case, the vacuole functions as a storage site of leucosin, a polymer of glucose and mannitol composed of 1:3 and 1:6 glucosidic linkages and a characteristic reserve product of the Chrysophyta (45). In mixed cultures, this same vacuole assumes an alternate function as a heterophagosome and, at this time, is probably less involved in storage of leucosin. As previously indicated, the posterior food vacuole, or, secondary phagosome, receives ingested organic material from the primary phagosome at the time of fusion of these two inclusions.

The characteristic shape of O. danica in axenic culture is pyriform with a distinctive elongate tail. However, when these cells feed on M. aeruginosa, and other microorganisms which we have examined, the chrysomonads assume an almost spherical shape. Retraction of the tail is witnessed at the time O. danica engulfs its first Microcystis at the anterior end of the cell. As the secondary phagosome expands with an increasing number of ingested blue-green algae, the chrysomonad becomes even more spherical. Bouck and Brown (10) have examined the biogenesis of microtubules in correlation with cell shape in O. danica and have suggested (11) that "the beak and rhizoplast sites could exert control over the position and timing of the appearance, the orientation, and the pattern of microtubule distribution...". When the authors exposed Ochromonas to colchicine or hydrostatic pressure (11), the microtubules disassembled, and the cells became spherical. When these artifical conditions were removed, the characteristic pyriform cell shape reappeared with concomitant assembly and orientation of microtubules. We suggest that the onset of endocytosis in O. danica also has a disruptive effect on the anterior microtubule nucleating sites, which causes initial disassembly of the cytoplasmic, skeletal system and subsequent retraction of the tail. The increased cellular turgor pressure established by the enlarged secondary phagosome perhaps causes additional disassembly of microtubules, and also has an inhibitory effect on regeneration of these structures. After digestion of the Microcystis cells, the chrysomonads slowly regain their pyriform shape, provided that they do not continue endocytotic activity. Resumption of this shape by O. danica is probably correlated with the reassembly, orientation, and distribution of microtubules controlled by the kinetobeak and rhizoplast nucleating sites of the cell.

Within two to five minutes after a Microcystis cell is encased by the secondary phagosome, a high degree of cytoplasmic activity, reminiscent of Brownian movement, adjacent to the upper surface of the digestive vacuole, is visible with the light microscope. Thin sections of this same region of the cytoplasm of O. danica, fixed at a comparable stage of intracellular digestion of M. aeruginosa, indicate that the particles observed with the light microscope are vesicles clustered in high concentration about the upper surface of the secondary phagosome (Figure 8). Both ultrastructural and cytochemical evidence indicates a close structural-functional interrelationship between these vesicles and the secondary phagosome. The vesicles, which appear to arise by a process of blebbing of the phagosomal membrane (Figure 13), contain acid phosphatase (Figure 24) as does the secondary phagosome itself (Figure 23). Closer observation of the organelles surrounding the secondary phagosomes revealed two additional kinds of vesicles: smaller vesicles of fairly uniform size which contain only diffuse electron-translucent material (Figures 13 and 18), and coated vesicles of variable diameter (Figures 10 through 12 and 18). De Duve (16) suggested that primary phagosomes, which arise as a result of endocytosis, are initially devoid of digestive enzymes. He further postulated, basing his concepts primarily on observations of polymorphonuclear leukocytes, that the primary phagosome receives its digestive enzymes, such as acid phosphatase, from primary lysosomes. The latter membrane-bounded organelles serve as storage sites for newly synthesized enzymes not yet involved in digestive events and are capable of fusing with the primary phagosome, releasing their contents into the vacuolar lumen, and thus, initiating the intracellular digestive process. Daems, Wisse, and Brederoo (14) identified another category of hydrolase-containing organelles in endocytotic cells as secondary lysosomes, which arise as blebs of the phagosomal membrane. The authors stated that these organelles contain undigested material, which has been translocated from the heterophagosome, and some of the products of digestion within the secondary lysosome eventually find their way out and into the cytoplasm without the escape of lysosomal enzymes. Primary lysosomes may fuse with secondary lysosomes, presumably to replenish the supply of digestive enzymes in the latter. We suggest that the large vesicles shown in Figures 8 and 9 are secondary lysosomes based on the facts that they occur in high concentration in the vicinity of the secondary phagosome (Figure 8), apparently arise from blebs of the phagosomal membrane (Figure 13), and contain acid phosphatase (Figure 24).

Daems _et al_. (14) also pointed out that lysosomes fuse only with phagosomes, or with each other, and that they are apparently incapable of fusing with membranes of other organelles. They proposed that this latter phenomenon, which is not well understood, exemplifies "fusion incompatibility". Of importance to this concept is the origin of the membranes encompassing each of the digestive organelles in the endocytotic cell. The membrane of the primary phagosome is of plasmalemma origin, while the ontogeny of the secondary phagosome is unknown. However, fusion of the two kinds of phagosomes demonstrates their membrane compatibility. Considerable evidence has accumulated for the origin of primary lysosomes from Golgi cisternae (14, 16, 18, 19, 32, 40, 51). Figure 22 demonstrates positive reaction for the test of the presence of acid phosphatase in the Golgi cisternae of _O. danica_. The dictyosome of the endocytotic chrysomonad appears to actively proliferate vesicles (Figure 21) and may account for most of the synthesis and compartmentalization of digestive enzymes in the cell. A sparsity of endoplasmic reticulum in _O. danica_, especially in the vicinity of the Golgi complex, led us to speculate earlier (12) that another cyto-membrane system, the nuclear envelope and vesicles arising from proliferation of its outer membrane, is also associated with this synthetic process (12). However, some ER lamellae are observed juxtaposed to the secondary phagosome (Figures 11, 14, 16 and 17). Trucket and Goulomb (52) observed in regions of root nodules of _Pisum sativum_ L. where bacterial parasitism occurs, that the process of lysosome formation from Golgi proliferation alone seemed insufficient considering the degree of lytic activity in the cells; and the authors, therefore, speculated that "endoplasmic reticulum then shunts the dictyosomes and directly buds phytolysosomes". Such a function may also be associated with

with ER in O. danica. Matile (35) concluded that the relatively large amounts of RNA found in isolated food vacuoles of plants are due to the presence of ribosomes. In thin sections of secondary phagosomes of O. danica (Figure 11), an abundance of ribosomes can be found in the matrix. It is proposed that phagosomes are capable of a certain degree of autonomous synthesis of lysosome enzymes.

Considerable evidence is available that coated veiscles play a role in endocytosis (14, 18, 20, 39). The origin of coated vesicles, however, is still rather controversial. Localized, invaginated regions of the plasmalemma of O. danica frequently demonstrated a coating of pentagonal and hexagonal subunits on the inner, cytoplasmic surface of the membrane (12, and Figure 18). It has been suggested that coated vesicles may arise by micropinocytosis of these regions of the cell membrane (14, 53) and function as endocytotic vesicles which may later fuse to form large food vacuoles (39). Franke and Herth (20) observed that the outer surfaces of coated indentations of the cell membranes of Dinobryon sertularia Ehr., another chrysomonad alga, were associated with diffuse, electron dense material which they suggested was of "glycoproteinaceous character". Similar observations are reported above for O. danica (Figure 18). Roth and Porter (44) had proposed earlier that coated vesicles may be specialized for cellular uptake of protein. Evidence is also available that coated vesicles arise directly from Golgi cisternae (12, 18, 22, 34). Holtzmann et al.(29) proposed that these vesicles function in transport of acid phosphatase from the dictyosome to heterophagosomes. We suggested earlier (12) that coated vesicles, arising from the Golgi, may subsequently fuse with other organelles such as the plasmalemma or secondary lysosomes (Figure 18) and "by some unknown mechanism deposit their coated surface upon the inner protein layer of the cell membrane". Kanaseki and Kadota (31) proposed that the coated surface of vesicles is an apparatus to control the "infolding and fissioning mechanism" of the plasmalemma. Their suggestion was based primarily on evidence that myosin-like material apparently comprises the subunits and this provided the necessary contractile properties to the membrane (6, 8). Perhaps the coated surfaces of secondary lysosomes is also associated with blebbing and fission of its membrane. It would appear, on the basis of current evidence, "that the group of coated vesicles comprises a functionally heterogeneous population" (14).

As suggested previously secondary lysosomes arise from blebbing of the membrane encompassing the secondary phagosome (Figures 11 and 13). These lysosomes, which commonly contain smaller vesicles as well as membrane fragments and heterogeneous material, may also be referred to as multivesicular bodies. However, caution is required in use of this terminology since evidence exists for a considerable variation in function and origin of structures generally referred to as mutlivesicular bodies (14). Our cytochemical studies of O. danica indicated that the multivesicular organelles surrounding the secondary phagosome contain acid phosphatase. Friend (21) suggested that these lysosome enzyme-containing structures may become multivesicular by penetration of small vesicles (e.g., primary lysosomes) through the outer membrane, or, by invagination and budding of the membrane itself. In O. danica, it is assumed that fusion between a number of functionally different organelles in the region of digestive activities is possible, necessitating a high degree of fusion compatibility between their respective membranes. For example, secondary lysosomes may fuse with primary lysosomes and with each other. Secondary lysosomes may arise from evaginations of the secondary phagosome and may subsequently fuse with the latter. The freeze-etch, surface fracture of the secondary phagosome in Figure 20 shows small circular scars which are reminescent of the circular pockets illustrated by the surface fractures of cell membranes of Tetrahymena pyriformis W. These membrane irregularities were attributed to the fusion of mucocysts (47, 48). Similar scars were demonstrated on the cell membrane of Chlorella Beij., which were considered to have resulted from fusion with Golgi vesicles (38).

Secondary lysosomes gradually gather indigestible products of endocytosis and finally become so filled with residues of digestion that they no longer participate in the segregation of material ingested by the cell (14). Such conversion of secondary lysosomes to residual bodies is indicated in Figure 17. These organelles and their contents may be gradually transformed into lipoidal deposits with a concomitant decrease in hydrolase enzymes contained by the lysosomal membrane (14). Accumulation of lipid deposits in O. danica has also been suggested to be indicative of aging (5, 49). The freeze-etch micrograph of O. danica in Figure 19, demonstrating evaginations of the cell membrane, is suggestive of a mechanism of exocytosis. Secretion of membranes extracellularly by O. danica has also been demonstrated in thin sections and has been proposed to be associated with an excretory process (4).

Our concepts of the various activities of phagosomes, lysosomes, and coated vesicles in O. danica are presented in a diagrammatic interpretation of intracellular digestion in Figure 26. More experimental evidence is required in order to validate or refute this interpretation of the sequence of events associated with endocytosis. One possible, future research approach is to use autoradiographic techniques involving labelled isoprenoids of the photosynthetic lamellae of M. aeruginosa, which appear to be rather slowly decomposed (Figures 13 through 15), and to trace the progressive dissipation of these radioactive products throughout the cytoplasm of O. danica.

ACKNOWLDGEMENTS

The authors are grateful to Dr. B. L. Prows of the Dow Chemical Company for donating strains of Microcystis aeruginosa and for providing advice and encouragement during the course of this project. Dr. H. C. Bold read the manuscript and offered suggestions. The project was supported by a Research Corporation Grant (BH-752) and a Dow Chemical Company Grant-in-Aid of Research to the senior author.

1. Aaronson, S. 1973a. Digestion of phytoflagellates. *In* Dingle, J. T. & Fell, H. B. (Ed.). Lysosomes in Biology & Pathology, Vol. 3, North Holland Pub., Amsterdam, pp. 18-37.
2. __________ 1973b. Particle aggregation and photo-autotrophy by Ochromonas. Arch. Mikrobiol. 92:39-44.
3. __________ & Baker, H. 1959. A comparative biochemical study of two species of *Ochromonas*. J. Protozool. 6:282-4.
4. __________, Behrens, U., Orner, R. & Haines, T. H. 1971. Ultrastructure of intracellular and extracellular vesicles, membranes, and myeline figures produced by *Ochromonas danica*. J. Ultrastruct. Res. 35:418-30.
5. __________ & Bensky, B. 1967. Effect of aging of a cell population on lipids and drug resistance in *Ochromonas danica*. J. Protozoll. 14:76-8.
6. Beams, H. W. & Kessel, R. G. 1968. The Golgi apparatus: structure and function. Int. Rev. Cytol. 23:209-76.
7. Bishop, C. T., Anet, E. F. L. J. & Gorham, P. R. 1959. Isolation and identification of the fast-death factor in *Microcystis aeruginosa* NRC-1. Can. J. Biochem. Physiol. 37:453-71.
8. Booij, H. L. 1966. Thoughts about the mechanism of membrane movements. *In* Warren, K. B. (Ed.). Intracellular Transport. Symposia of the Int. Soc. Cell Biol., Vol. 5, Academic Press, New York, pp. 301-17.
9. Bouck, G. B. 1965. Fine structure and organelle associations in brown algae. J. Cell Biol. 26:523-37.
10. __________ & Brown, D. L. 1973. Microtubule biogenesis and cell shape in *Ochromonas*. I. The distribution of cytoplasmic and mitotic microtubules. J. Cell Biol. 56:340-59.

11. Brown, D. L. & Bouck, G. T. 1973. Microtubule biogenesis and cell shape in Ochromonas. II. The role of nucleating sites in shape development. J. Cell Biol. 56:360-78.

12. Cole, G. T. & Wynne, M. J. 1973. Nuclear pore arrangement and structure of the Golgi complex in Ochromonas danica (Chrysophyceae). Cytobios In press.

13. Daems, W. T. & Van Rijssel, T. G. 1961. The fine ssructure of the peribiliary dense bodies in mouse liver tissue. J. Ultrastruct. Res. 5:263-90.

14. __________, Wisse, E. & Brederoo, P. 1972. Electron microscopy of the vacuolar apparatus. In Dingle, J. T. (Ed.). Lysosomes: A laboratory handbook. North Holland Pub., Amsterdam, pp. 159-99.

15. Daley, R. J., Morris, G. P. & Brown, S. R. 1973. Phagotrophic ingestion of a blue-green algae by Ochromonas. J. protozool. 25:58-61.

16. De Duve, C. 1963. The lysosome concept. In de Reuck, A. V. S. & Cameron, M. P. (Eds.). Ciba Foundation Symposium on Lysosomes. Little, Brown, Boston, pp. 1-35.

17. Drews, G. 1973. Fine structure and chemical composition of the cell envelopes. In Carr, N. G. & Whitton, B. A. (Eds.). The Biology of Blue-Green Algae. Botanical Monographs, Vol. 9, Univ. of Calif. Press, Berkeley, pp. 99-116.

18. Erlandsen, S. L. & Chase, D. G. 1972. Paneth cell function: phagocytosis and intracellular digestion of intestional microorganisms. II. Spiral microorganism. J. Ultrastruct. Res. 41:319-33.

19. Esteve, J. C. 1970. Distribution of acid phosphatase in Paramecium caudatum: its relations with the process of digestion. J. Protozool. 17:24-35.

20. Franke, W. W. & Herth, W. 1973. Cell and lorica fine structure of the chrysomonad alga, Dinobryon sertularia Ehr. (Chrysophyceae). Arch. Mikrobiol. 91:323-44.

21. Friend, D. S. 1969. Cytochemical staining of multivesicular body and Golgi vesicles. J. Cell Biol. 41:269-79.

22. __________ & Farquhar, M. G. 1967. Functions of coated vesicles during protein absorption in the rat vas deferens. J. Cell Biol. 35:357-76.

23. Gibbs, S. P. 1962. Nuclear envelope-chloroplast relationships in algae. J. Cell Biol. 14:433-44.

24. __________ 1962. Chloroplast development in Ochromonas danica. J. Cell Biol. 15:343-61.

25. Gomori, G. 1952. Microscopic Histochemistry: Principles and practice. Chicago Press, 273 pp.

26. Gorham, P. R. 1964. Toxic algae as a public health hazard. Jour. Am. W. W. Assoc. 56:1481-8.

27. __________ 1964. Toxic algae. In Jackson, E. F. (Ed.). Algae and Man. Plenum Press, New York, pp. 307-36.

28. Herman, L., Eber, L. & Fitzgerald, P. J. Liver cell degeneration with ethionine administration. In Bresse, S. S. Jr. (Ed.). Electron Microscopy. Fifth International Congress for Electron Microscopy, Vol. 2, Academic Press, New York, p. VV-6.

29. Holtzman, E., Novikoff, A. B. & Villaverde, H. 1967. Lysosomes and GERL in normal and chromatolytic neurons of the rate ganglion nodosum. J. Cell Biol. 33:419-35.

30. Jacques, P. J. 1963. Endocytosis. In Dingle, J. T. & Fell, H. B. (Eds.). Lysosomes in Biology & Pathology. Frontiers of Biology, Vol. 14B, North Holland Pub., Amsterdam, pp. 395-420.

31. Kanaseki, T. & Kadota, K. 1969. The "vesicle in a basket." A morphological study of the coated vesicle isolated from the nerve endings of the guinea pig brain, with special reference to the mechanism of membrane movements. J. Cell Biol. 42:202-20.

32. Kazama, F. 1973. Ultrastructure of *Thraustochytrium* sp. zoospores. III. Cytolysomes and acid phosphatase distribution. Arch. Mikrobiol. 89:95-104.

33. Konst, H., McKercher, P. D., Gorham, P. R., Robertson, A. & Howell, J. 1965. Symptoms and pathology produced by toxic *Microcystis aeruginosa* NRC-1 in laboraotry and domestic animals. Can J. Comp. Med. & Vet. Sci. 29:221-8.

34. Lane, N. J. 1968. Distribution of phosphatases in the Golgi region and associated structures of the thoracic ganglionic neurons in the grasshopper, *Melanoplus differentialis*. J. Cell Biol. 37:89-104.

35. Matile, PH. 1969. Plant lysosomes. *In* Dingle, J. T. & Fell, H. B. (Eds.). Lysosomes in Biology and Pathology I. Frontiers of Biology, Vol. 14A, North Holland Pub., Amsterdam. pp. 406-30.

36. Meyer, D. H. & Aaronson, S. 1973. Evidence for the secretion by *Ochromonas danica* of an acid hydrolase into its environment. J. Phycol. 9(Suppl.):20(Abstrct).

37. Miller, M. M. and Lang, N. J. 1971. The effect of aging on thylakoid configuration and granular inclusions in *Gloeotrichia*. *In* Parker, B. C. & Brown, R. M. Jr. (Eds.). Contributions in Phycology, Allen Press, Kansas, pp. 53-8.

38. Mühlethaler, K. 1967. Ultrastructure and formation of plant cell walls. Ann. Rev. Plant Physiol. 18:1-24.

39. Munch, R. 1970. Food uptake by endocytosis in _Opalina_ _ranarum_. Cytobiologie 2:108-22.

40. Novikoff, A. B. 1963. Lysosomes in the physiology and pathology of cells: contributions of staining methods. _In_ de Reuck, A. V. S. & Cameron, M. P. (Eds.). Ciba Foundation Symposium on Lysosomes, Little, Brown, Boston, pp. 36-73.

41. Okino, T. 1973. Studies on the blooming of _Microcystis_ _aeruginosa_. Jap. J. Bot. 20:381-402.

42. Pankratz, H. S. & Bowen, C. C. 1963. Cytology of blue-green algae. I. The cells of _Symplica_ _muscorum_. Am. J. Bot. 50:387-99.

43. Pringsheim, E. G. 1952. On the nutrition of _Ochromonas_. Quart. J. Microscop. Sci. 93:71-96.

44. Roth, T. F. & Porter, K. R. 1964. Yolk protein uptake in the oocyte of the mosquito _Aedes_ _aegypti_ L. J. Cell Biol. 20:313-32.

45. Round, F. E. 1965. The Biology of the Algae. Arnold Pub., London, 269 pp.

46. Salomon, J. C. 1962. Modifications des cellules du parenchyme hepatique du rat sous l'effet de la thioacetamide. J. Ultrastruct. Res. 7:293-307.

47. Satir, B., Schooley, C. & Satir, P. 1973. Membrane fusion in a model system. J. Cell Biol. 56:153-76.

48. __________ 1972. Membrane reorganization during secretion in _Tetrahymena_. Nature 235:53-4.

49. Schuster, F. L., Hershenov, B. & Aaronson, S. 1968. Ultrastructural observations on aging of stationary cultures and feeding in Ochromonas. J. Protozool. 15:335-46.

50. Slankis, T. & Gibbs, S. P. 1972. The fine structure of mitosis and cell division in the chrysophycean alga Ochromonas danica. J. Phycol. 8:243-56.

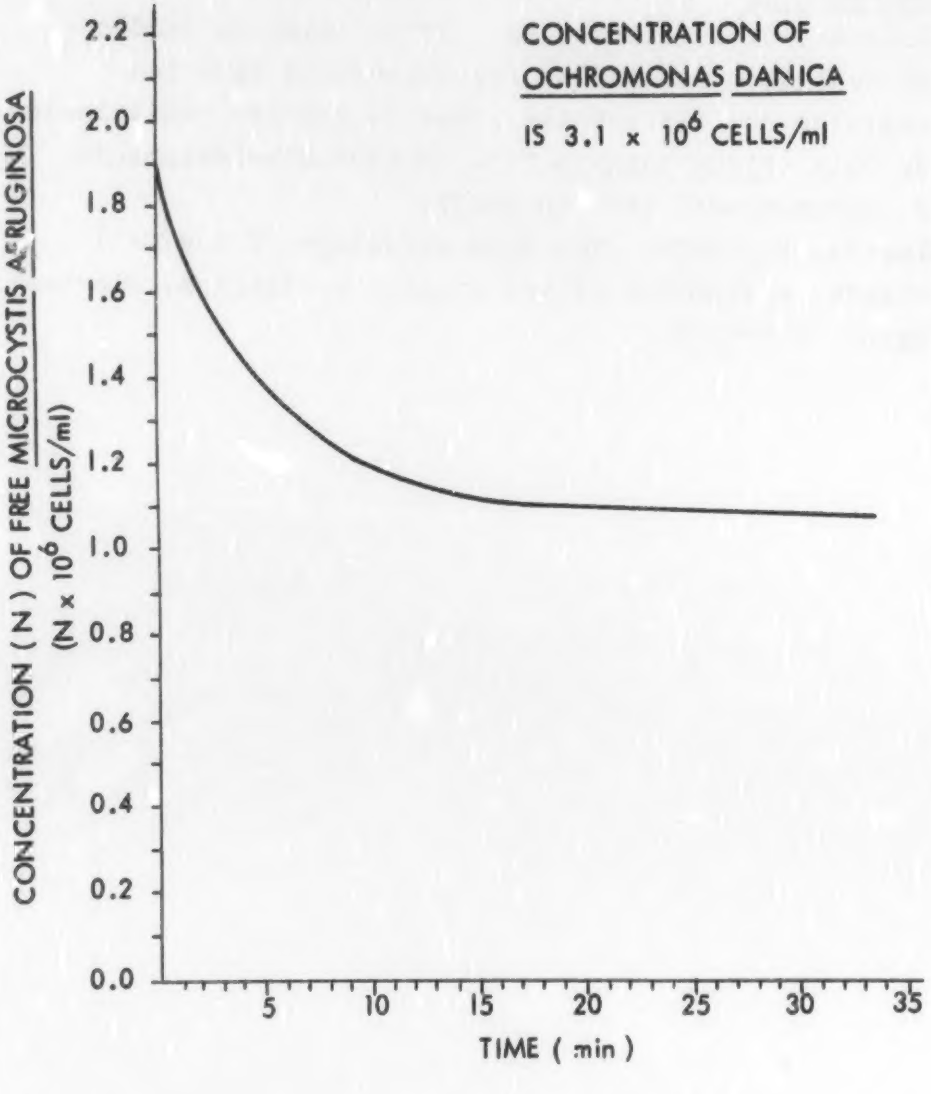
51. Stoltze, H. J., Lui, N. S. T., Anderson, O. R. & Roels, O. A. 1969. The influence of the mode of nutrition on the digestive system of Ochromonas malhamensis. J. Cell Biol. 43:396-409.

52. Trucket, G. & Coulomb, PH. 1973. Mise en évidence et évolution de système Phytolysosomal dans les cellules des differéntes zones de nodules radicularies de Pois (Pisum sativum L.). Notion d'hétérophagie. J. Ultrastruct. Res. 43:36-57.

53. Yamada, E. 1955. The fine structure of the gall bladder epithelium of the mouse. J. Biophys. Biochem. Cytol. 1:445-58.

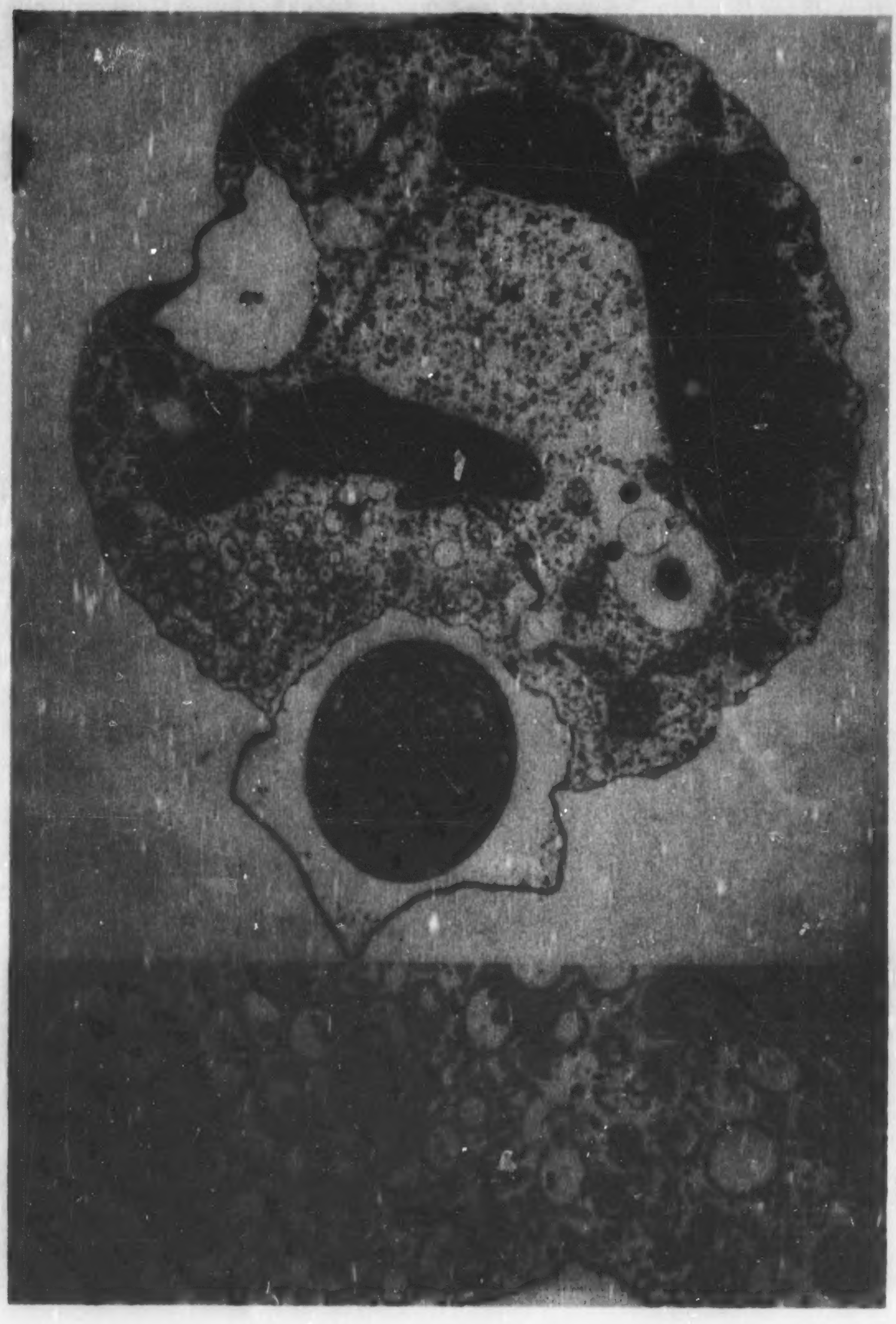
TABLE I

Cole and Wynne, 1973



Figures 1-7

Figures 8-10



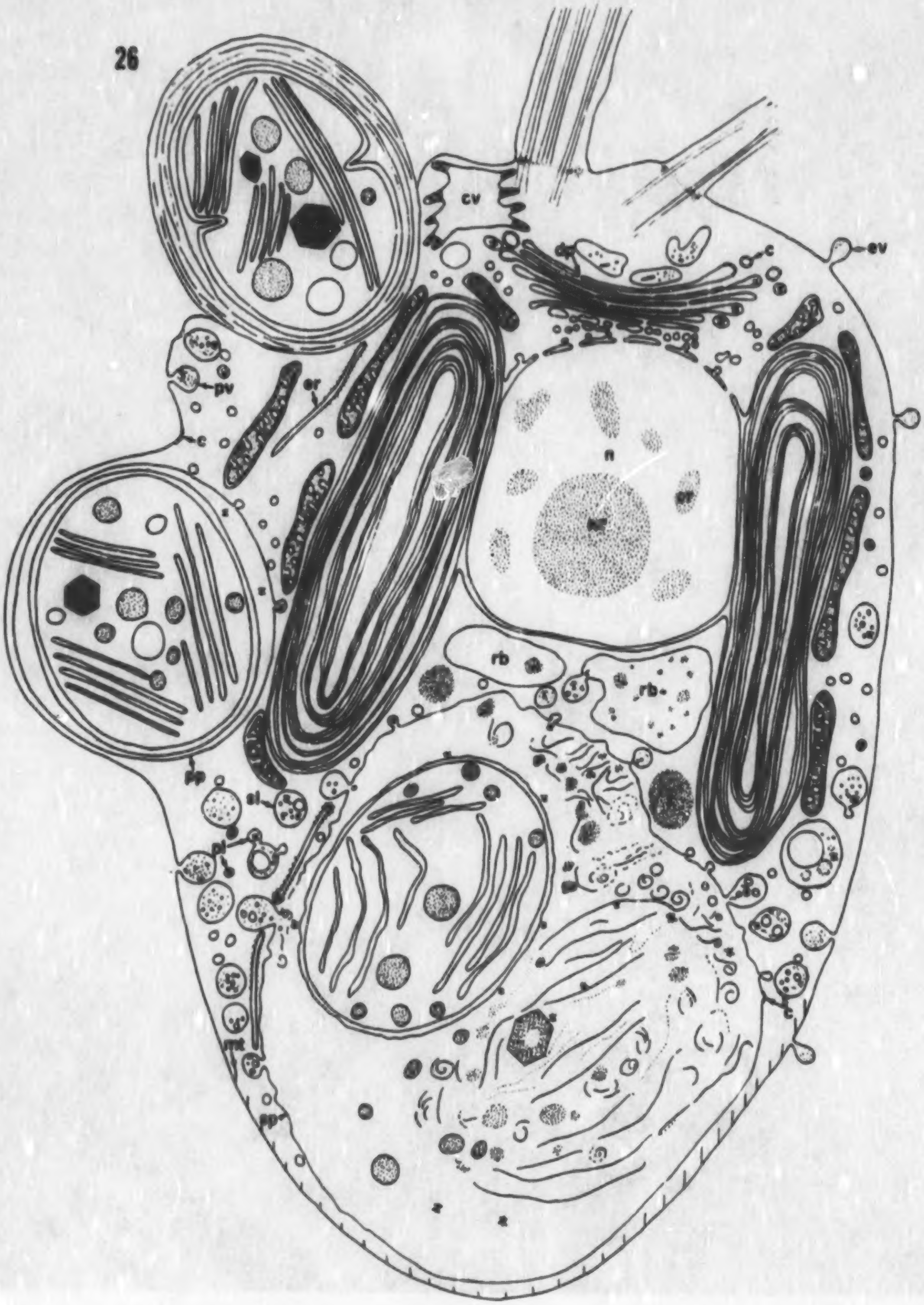
Figures 11-15

Figures 16-19

Figures 20-25

Figure 26

26


cv
dp
c
ev
pv
er
c
n
rb
rb
pp
sl
pl
mt
pp
c

LEGENDS FOR FIGURES

Figures 1-4	Interference contrast (Nomarski) light micrographs of *O. danica* at various stages of endocytosis of *M. aeruginosa*. Arrow indicates free-living *M. aeruginosa*. cv, contractile vacuole; F, flagellum; pp, primary phagosome; sp, secondary phagosome. Figures 1-4, X 2,000.
Figures 5-7	Freeze-etch micrographs of *O. danica* (Figure 5) and *M. aeruginosa* (Figures 6 and 7). Figure 5 illustrates a secondary phagosome containing partially digested *Ochromonas* (O) and *Microcystis* (M) cells. The free-living *M. aeruginosa* cell (Figure 6) contains a crystalloid (C), which at higher magnification (Figure 7), demonstrates a distinct lattice (arrows). cm, cell membrane; L, lipid droplet; pl, photosynthetic lamella; V, gas vacuole. Figure 5, X 14,740. Figure 6, X 32,000. Figure 7, 67,200.
Figures 8-10	Thin sections of *O. danica* which contains a *M. aeruginosa* cell in its posterior, secondary phagosome (Figure 8). Figure 9 is a higher magnification of secondary lysosomes concentrated just above secondary phagosome. A coated vesicle (arrow in Figure 9 and 10) is also shown. Arrows in Figure 8 indicate continuities between nuclear envelope and chloroplast ER. D, dictyosome; N, nucleus. Figure 8, X 14,740. Figure 9, X 37,200. Figure 10, X 111,600

Figures 11-15 Thin sections of secondary phagosomes and surrounding organelles of *O. danica* at progressive stages of digestion of *M. aeruginosa*. A partially coated vesicle (arrow in Figure 11) is shown at higher magnification in Figure 12. Arrows in Figure 13 show small vesicles, suggested to be primary lysosomes, juxtaposed to the phagosomal membrane and membranes of larger vesicles, identified as secondary lysosomes. Figure 15 shows a late stage of the intracellular digestion of *M. aeruginosa*. Arrows indicate fragments of membranes which are partially detached from the decomposed cellular material. Arrowheads indicate detached loops of membrane, the ends of which have fused. er, endoplasmic reticulum; mf, myeline-figure. Figure 11, X 18,000. Figure 12, X 36,600. Figure 13, X 50,000. Figure 14, X 24,600. Figure 15, X 67,500.

Figures 16-17 Thin sections of secondary phagosomes and surrounding organelles at progressive stages of digestion of *M. aeruginosa*. rb. residual body. Figure 16, X 30,000. Figure 17, X 21,600.

Figure 18 Thin section through cytoplasm of *O. danica* adjacent to secondary phagosome showing partially coated vesicles, and coated region of cell membrane (ccm). Arrows indicate primary lysosomes. X 40,800.

Figure 19	Freeze-etch, cross-fracture of *O. danica* showing evaginations of the cell membrane (arrows). Ch, chloroplast. X 23,500.
Figure 20	Freeze-etch, surface fracture of the cell membrane and secondary phagosome of *O. danica*. Arrows indicate circular scars on the fractured, phagosomal membrane. X 11,390.
Figure 21	Freeze-etch cross-fracture of the dictyosome of *O. danica*. X 23,200.
Figures 22-24	Thin sections of endocytotic *O. danica* cells subjected to the modified Gomori reaction which is used to localized acid phosphatase activity. The latter is indicated by the electron-dense deposits in Golgi cisternae (Figure 22), in the secondary phagosome associated with partially digested *M. aeruginosa* cell (Figure 23), and in secondary lysosomes (Figure 24). Figure 22, X 26,400. Figure 23, X 22,400. Figure 24, X 25,200.
Figure 25	Thin section of *O. danica* showing partially digested bacterial cells enclosed by secondary phagosome and intact bacteria outside the phagocyte. X 17,390.
Figure 26	Diagrammatic interpretation of cellular processes associated with endocytosis of *M. aeruginosa* by *O. danica*.

Table I	Graphical demonstration of the rate of endocytosis which is interpreted as the rate of decrease of free M. aeruginosa cells in the presence of a known concentration of O. danica.

APPENDIX B

DETAILED SCREENING DATA

COMPOUND ACTIVITY OF SELECTED ALGAECIDAL COMPOUNDS AGAINST *Anabaena* WHICH WAS CONTAMINATED WITH AN UNKNOWN SPECIES OF FILAMENTOUS GREEN ALGAE AT VARIOUS CONCENTRATIONS

Serial Number	Compound Name	Concentration ppm	Initial Relative Intensity	Final Relative Intensity	Percent Control
23	2,5-Dichloro-3,4-dinitrothiophene	3.2	.20	.058	79
		1.6	.20	.058	79
		0.8	.20	.054	81
73	p-Chlorophenyl-2-thienyliodonium-chloride	3.2	.20	.030	89
		1.6	.20	.037	87
		0.8	.20	.052	80
	Control	---	.20	.28	--

COMPOUND ACTIVITY - SUPPLEMENTARY SCREENING DATA

Anabaena flos-aquae

Serial Number	Name of Compound	Initial Relative Intensity	Final Relative Intensity	Percent Control
74	(p-Bromophenyl)-2-thienyl iodonium chloride			
	1.6 ppm	.23	.04	100
	0.8 ppm	.27	.05	95
	0.4 ppm	.18	.37	59
78	2-Thienyl-p-tolyliodonium chloride			
	1.6 ppm	.28	.06	94
	0.8 ppm	.19	.32	65
	0.4 ppm	.18	.47	48
82	(o-Chlorophenyl)-2-thienyl iodonium chloride			
	1.6 ppm	.36	.05	95
	0.8 ppm	.32	.06	94
	0.4 ppm	.22	.08	92
96	2,5-Dibromo-3,4-dinitrothiophene			
	1.6 ppm	.27	.04	100
	0.8 ppm	.26	.04	100
	0.4 ppm	.23	.05	95

Serial Number	Name of Compound	Initial Relative Intensity	Final Relative Intensity	Percent Control
98	Tetrachlorothiophene			
	1.6 ppm	.23	.70	23
	0.8 ppm	.21	.70	23
	0.4 ppm	.19	.78	14
	Control			
	No. 1	.19	.93	
	No. 2	.18	.97	
	No. 3	.18	.82	

COMPOUND ACTIVITY SCREENING OF SELECTED ALGAECIDAL COMPOUNDS AGAINST Anabaena flos-aquae AT 0.8 PPM

Serial Number		Initial Relative Intensity	Final Relative Intensity	Percent Control
121	N'-(3-(1,1-Dimethylethy)-4-nitrophenyl)-N,N-dimethyl urea	(1) .29 (2) .31	(1) .66 (2) .62	9
124	N-(4-Butyl-2-nitrophenyl)-acetamide	(1) .19 (2) .19	(1) .73 (2) .69	0
163	N'-(4-((3-Chloro-2-pyridinyl)oxy)phenyl)-N,N-dimethyl urea	(1) .30 (2) .28	(1) .90 (2) .90	0
164	N'-(4-Ethylphenyl)-N,N-dimethyl urea	(1) .47 (2) .43	(1) .70 (2) .71	0
165	N'-(4-((3,5-Dichloro-2-pyridinyl)oxy)phenyl)-N,N-dimethyl urea	(1) .48 (2) .44	(1) 1.10 (2) .95	0
	Control	(1) .21 (2) .20	(1) .70 (2) .70	-

COMPOUND ACTIVITY OF SELECTED ALGAECIDAL COMPOUNDS AGAINST Anabaena flos-aquae AT 0.8 PPM

4-day period

Serial Number	Name of Compound	Initial Relative Intensity	Final Relative Intensity	Percent Control
126	7,8-Dihydro-6H-pyrrolo(1,2-e) purin-4-01	(1) .29	(1) 1.05	0
		(2) .31	(2) 1.0	
127	2-Tert-butyl-4-nitrophenol	(1) .28	(1) .75	12
		(2) .28	(2) .91	
136	2,2'-(1,2-Ethenediyl)bisbenzoxazole	(1) .29	(1) .08	91
		(2) .32	(2) .10	
	Control	(1) .30	(1) .98	--
		(2) .29	(2) .89	

COMPOUND NUMBER 136 - 2,2'-(1,2-Ethenediyl)bisbenzoxazole AGAINST *Microcystis aeruginosa* AND *Anabaena flos-aquae* AT VARIOUS CONDITIONS

Concentration ppm	Anabaena flos-aquae			Microcystis aeruginosa		
	Initial Relative Intensity	Final Relative Intensity	Percent Control	Initial Relative Intensity	Final Relative Intensity	Percent Control
1.6	.35	.04	100	.28	.066	100
1.6'	.36	.05		.25	.066	
0.8	.27	.069	94	.22	.070	94
0.8'	.27	.080		.22	.072	
0.4	.26	.27	80	.21	.066	94
0.4'	.26	.26		.21	.070	
0.2	.25	.92	35	.21	.11	87
0.2'	.24	.83		.21	.20	
Control	.26	1.4	--	.21	1.1	--
Control'	.28	1.3		.21	1.2	

LABORAORY SCREENING TESTS
ALGAECIDAL ACTIVITY OF COMPOUND NO. 136
(2,2'-(1,2-Ethenediyl)bis-benzoxazole)
AT VARIOUS CONCENTRATIONS OVER AN EXTENDED TEST PERIOD

Anabaena flos-aquae

Concentration	0-days R.I.	0+4 days R.I.	% Control	0+21 days R.I.	% Control
0.8 ppm	0.20	0.11	70	1.1	76
0.4 ppm	0.20	0.16	57	1.4	68
0.2 ppm	0.20	0.19	52	2.7	41
0.1 ppm	0.20	0.27	33	3.1	28
Control No. 1	0.20	0.39	--	5.2	--
Control No. 2	0.20	0.40	--	4.1	--

Microcystis aeruginosa

0.8 ppm	0.20	0.51	90	2.20	21
0.4 ppm	0.20	0.13	76	1.95	30
0.2 ppm	0.20	0.37	31	2.55	<10
0.1 ppm	0.20	0.48	10	2.05	23
Control No. 1	0.20	0.54	--	2.90	--
Control No. 2	0.20	0.54	--	2.70	--

COMPOUND ACTIVITY SCREENING OF SELECTED ALGAECIDAL COMPOUNDS AGAINST *Anabaena flos-aquae* AT 0.8 PPM

Serial Number	Name of Compound	Initial Relative Intensity	Final Relative Intensity	Percent Control
146	5-Butyl-2-methyl-1H-benzimidazole	(1) .40 (2) .40	(1) .40 (2) .40	0
147	Pentachlorophenol, compound with 2-(2,4,5-Trichlorophenoxy)ethanamine (1:1)	(1) .24 (2) .24	(1) .79 (2) .80	0
148	N'-(4-((6-Chloro-4-trifluoromethyl)-2-pyridinyl) oxy)-N,N-dimethyl urea	(1) .21 (2) .21	(1) 1.50 (2) 1.10	0
149	5-Nitro-2-thiophenecarboxaldehyde, oxime	(1) .28 (2) .28	(1) .76 (2) .79	0
150	N-(4-((6-Chloro-2-pyridinyl) oxy)phenyl) acetamide	(1) .33 (2) .34	(1) 1.00 (2) 1.00	0
	Control	(1) .24 (2) .25	(1) .73 (2) .75	-

COMPOUND ACTIVITY SCREENING OF SELECTED ALGAECIDAL COMPOUNDS AGAINST *Anabaena flos-aquae* AT 0.8 PPM

Serial Number	Name of Compound	Initial Relative Intensity	Final Relative Intensity	Percent Control
151	N-(4-((6-Chloro-2-pyridinyl) oxy)phenyl)-N'-methyl urea	(1) .23 (2) .23	(1) 1.20 (2) 1.00	0
152	N'-(4-((2,6-Dichloro-4-pyridinyl)oxy)phenyl)-N,N-dimethyl urea	(1) .66 (2) .64	(1) 1.60 (2) 1.60	0
153	2,5-Bis((4-methylphenyl) sulfonyl)-3,4-dinitrothiophene	(1) .28 (2) .26	(1) .06 (2) .75	8
154	N-(3-Chlorophenyl)-2-isoxazolidinecarboxamide	(1) .34 (2) .30	(1) .90 (2) .90	0
155	N-(4-Chlorophenyl)-2-isoxazolidinecarboxamide	(1) .27 (2) .26	(1) .70 (2) .51	18
	Control	(1) .24 (2) .25	(1) .73 (2) .75	--

COMPOUND ACTIVITY SCREENING OF SELECTED ALGAECIDAL COMPOUNDS AGAINST *Anabaena flos-aquae* AT 0.8 PPM

Serial Number	Name of Compound	Initial Relative Intensity	Final Relative Intensity	Percent Control
156	3-(Acetyloxy)-4-bromobutanoic acid, methyl ester	(1) .24 (2) .25	(1) .60 (2) .80	6
157	N'-(4-Acetylphenyl)-N,N-dimethyl urea	(1) .31 (2) .30	(1) .83 (2) .80	0
158	N,N-Dimethyl-N'-(4-((6-(methylthio)-2-pyridinyl)-oxy)phenyl) urea	(1) .61 (2) .61	(1) 1.20 (2) 1.00	0
159	N'-(4-((6-Chloro-2-pyridinyl)oxy)phenyl)-N,N-diethyl urea	(1) .64 (2) .65	(1) .90 (2) 1.30	0
160	2-(2,4-Dichlorophenoxy)-3-nitropyridine	(1) .27 (2) .30	(1) .72 (2) .75	2
	Control	(1) .24 (2) .25	(1) .73 (2) .75	-

COMPOUND ACTIVITY SCREENING OF SELECTED ALGAECIDAL COMPOUNDS AGAINST *Anabaena flos-aquae* AT 0.8 PPM

Serial Number	Name of Compound	Initial Relative Intensity	Final Relative Intensity	Percent Control
161	Tris(dimethylamino)(hydroxyphenyl=methyl) phosphonium, hydroxide, inner salt	(1) .28 (2) .26	(1) .68 (2) .67	10
162	N'-(4-((6-Chloro-2-pyrazinyl)oxy)phenyl)-N,N-dimethyl urea	(1) .45 (2) .43	(1) .85 (2) .88	0
	Control	(1) .24 (2) .25	(1) .73 (2) .75	-

COMPOUND ACTIVITY SCREENING OF SELECTED ALGAECIDAL COMPOUNDS AGAINST *Anabaena flos-aquae* AT 0.8 PPM

Serial Number	Name of Compound	Initial Relative Intensity	Final Relative Intensity	Percent Control
166	4-Amino-6-(1,1-dimethyl=ethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one	(1) .55 (2) .50	(1) .90 (2) .90	13
167	1H-Imidazol-2-ylphenyldiazene	(1) .42 (2) .39	(1) 1.10 (2) 1.14	0
168	2-Chloro-6-(4-methoxyphenoxy) pyridine	(1) .45 (2) .52	(1) .94 (2) 1.07	0
169	N'-(4-(2,6-dinitro-4-(trifluoromethyl)phenoxy)=phenyl)-N,N-dimethyl urea	(1) .52 (2) .50	(1) 1.05 (2) 1.05	0
	Control	(1) .21 (2) .50	(1) .70 (2) .70	-

COMPOUND ACTIVITY SCREENING OF SELECTED ALGAECIDAL COMPOUNDS AGAINST *Anabaena flos-aquae* AT 0.8 PPM

Serial Number	Name of Compound	Initial Relative Intensity	Final Relative Intensity	Percent Control
170	2-(4-Chlorophenyl)-2,3,5,6-tetrahydroimidazo(2,1-b)-thiazole, monohydrochloride	(1) .20 (2) .20	(1) .63 (2) .56	14
171	2,3,5,6-Tetrahydro-2-(2-naphthalenyl)-imidazo(2,1-b), monohydrochloride	(1) .20 (2) .19	(1) .62 (2) .61	11
172	2-Hydroxy-N-phenyl-3-pyridinecarboxamide	(1) .20 (2) .23	(1) .64 (2) .75	4
173	N'-(3-Chlorophenyl-N-methoxy-N-methyl urea	(1) .36 (2) .28	(1) .93 (2) .82	0
	Control	(1) .21 (2) .20	(1) .70 (2) .70	-

COMPOUND ACTIVITY OF SELECTED ALGAECIDAL COMPOUNDS AGAINST *Anabaena flos-aquae* AT 0.8 PPM

Four-Day Test

Serial Number	Compound Name	Initial Relative Intensity	Final Relative Intensity	Percent Control
177	1-(2,4,5-Trichlorophenoxy)=thiocyanic acid, ethyl ester	(1) .20 (2) .20	(1) .27 (2) .32	65
178	2-((2-(Dimethylamino)ethyl)amine)3,4-dihydro-1(2H) isoquinolinone, dihydrochloride	(1) .20 (2) .20	(1) 1.05 (2) .96	0
179	2-Phenyl-5H-(1,2,4)triazolo(1,5-b)-isoindole	(1) .20 (2) .20	(1) 1.0 (2) 1.30	0
180	2-(3-Methylphenyl)-5H-(1,2,4)triazolo(1,5-b)isoindole	(1) .20 (2) .20	(1) .90 (2) .79	0
	$CuSO_4 \cdot 5H_2O$	(1) .20 (2) .20	(1) .10 (2) .15	85
	Control	(1) .20 (2) .20	(1) .95 (2) .75	--

COMPOUND ACTIVITY OF SELECTED ALGAECIDAL COMPOUNDS AGAINST *Anabaena flos-aquae* AT 0.8 PPM

Four-Day Test

Serial Number	Compound Name	Initial Relative Intensity	Final Relative Intensity	Percent Control
183	3-(4-(1,1-Dimethyl=ethyl)phenyl)-2,3,5,6-tetrahydroimidazo(2,1-b)thiazole	(1) .20 (2) .20	(1) 1.10 (2) 1.10	0
184	Phenyl-2-thienyl methanone, o-((methyl=amino)carbonyl)oxime	(1) .20 (2) .20	(1) .55 (2) .68	28
185	Bis((1,1'-biphenyl)-4-yl)-ethanedione	(1) .20 (2) .20	(1) .95 (2) .81	0
186	((3,5-Dichloro-6-fluoro-2-pyridinyl)oxy)methyl ester thiocyanic acid	(1) .20 (2) .20	(1) .30 (2) .31	65
	$CuSO_4 \cdot 2H_2O$	(1) .20 (2) .20	(1) .10 (2) .15	85
	Control	(1) .20 (2) .20	(1) .95 (2) .75	--

COMPOUND ACTIVITY OF SELECTED ALGAECIDAL COMPOUNDS AGAINST *Anabaena flos-aquae* AT 0.8 PPM

Four-Day Test

Serial Number	Compound Name	Initial Relative Intensity	Final Relative Intensity	Percent Control
176	1,2-Dichloro-4-(isothiocyanatomethoxy)benzene	(1) .20 (2) .20	(1) .10 (2) .09	97
182	((4,5-Dimethoxy-1-2-phenylene)-bis=(imino(thioxomethylene)))bis-carbamic acid, dimethyl ester	(1) .20 (2) .20	(1) .70 (2) .88	0
193	N'-(4-((6-Chloro-2-pyridinyl)oxy)-3-(trifluoromethyl)phenyl)-N,N-dimethyl urea	(1) .20 (2) .20	(1) .40 (2) .45	41
194	N,N-Dimethyl-N'-(4-((6-(trifluoromethyl)-2-pyridinyl)thio)phenyl)urea	(1) .20 (2) .20	(1) .37 (2) .40	61
	Control	(1) .20 (2) .20	(1) .68 (2) .75	--

COMPOUND ACTIVITY OF SELECTED ALGAECIDAL COMPOUNDS AGAINST *Anabaena flos-aquae* AT 0.8 PPM

Four-Day Test

Serial Number	Compound Name	Initial Relative Intensity	Final Relative Intensity	Percent Control
187	2-(4-Ethoxyphenyl)2,3,5,6-tetrahydroimidazo(2,1-b)thiazole	(1) .20 (2) .20	(1) .88 (2) .84	5
188	N-(1-(4-Bromo-2,5-dichlorophenoxy)-2,2,2-trichloroethyl)formamide	(1) .20 (2) .20	(1) 1.40 (2) 1.30	0
189	N'-(4-Ethyl-3-nitrophenyl)N,N-dimethyl urea	(1) .20 (2) .20	(1) .85 (2) .95	3
190	4-((3,5,6-Trichloro-2-pyridinyl)-oxy)phenol	(1) .20 (2) .20	(1) 1.00 (2) 1.00	0
	$CuSO_4 \cdot 5H_2O$	(1) .20 (2) .20	(1) .08 (2) .08	91
	Control	(1) .20 (2) .20	(1) .88 (2) .92	--

COMPOUND ACTIVITY SCREENING TESTS OF ALGAECIDAL COMPOUND AGAINST *Anabaena flos-aquae* AND *Microcystis aeruginosa* AT 0.8 PPM

Serial Number	Compound Name	Microcystis Initial Relative Intensity	Microcystis Final Relative Intensity	Microcystis Percent Control	Anabaena Initial Relative Intensity	Anabaena Final Relative Intensity	Anabaena Percent Control
192	N'-(4-((2-Chloro-6-methyl-4-pyrimidinyl)-oxy)phenyl)-N,N-dimethyl urea	.20	.53	41	.20	.92	0
		.20	.53		.20	.91	
197	1-(3,3-Dichloro-1-methylenebutyl)-3,5-dimethyl benzene	.20	.66	14	.20	.52	20
		.20	.62		.20	.52	
	Control	.20	.74	--	.20	.65	--

COMPOUND ACTIVITY OF SELECTED ALGAECIDAL COMPOUNDS AGAINST *Anabaena flos aquae* AT 0.8 PPM

Four-Day Test

Serial Number	Compound Name	Initial Relative Intensity	Final Relative Intensity	Percent Control
191	N'-(3-Chloro-4-((6-chloro-2-pyridinyl)oxy)phenyl)-N,N-dimethyl urea	-(1) .20 (2) .20	(1) .74 (2) .74	18
195	N-(1-Methylethyl)-4-phenoxybenzenamine	(1) .20 (2) .20	(1) 1.50 (2) 1.40	0
196	N'-(4-((6-Bromo-2-pyridinyl)thio)phenyl)-N,N-dimethyl urea	(1) .20 (2) .20	(1) .77 (2) .96	0
	$CuSO_4 \cdot 5H_2O$	(1) .20 (2) .20	(1) .08 (2) .08	91
	Control No. 1	(1) .20 (2) .20	(1) .88 (2) .92	--
	Control No. 2	(1) .20 (2) .20	(1) .68 (2) .75	--

LABORATORY SCREENING TESTS
ALGAECIDAL ACTIVITIES OF SELECTED TEST COMPOUNDS AGAINST *Anabaena flos-aquae* FOR EXTENDED PERIODS

Test Concentration — 0.8 ppm

Compound Number and Name	0-day R.I.*	0+4 days R.I.*	% Control	0+7 days R.I.*	0+10 days	% Control
198 p-(p-Nitrophenylthio) phenol	0.20	0.53	0	0.88	0.87	22
199 1-((4-Nitrophenyl)methyl) piperidine	0.20	0.49	0	0.87	1.14	0
200 (5-Chloro-2,4-dimethoxyphenyl)-carbamic acid, 2,4,5-trichlorophenyl ester	0.20	0.56	0	1.12	1.17	0
201 2,4-Dibromo-3-methyl-6-nitrophenol	0.20	0.52	0	1.05	1.15	0
202 2,4-Dichloro-3-methyl-6-nitrophenol	0.20	0.53	0	1.02	1.16	0
203 N-Bromophenyl-2-chloroacetamide	0.20	0.53	0	1.00	1.14	0
204 4-((6-Fluoro-2-pyridinyl)=thio)phenol	0.20	0.58	0	0.98	1.15	0
205 1-Chloro-2-(methylsulfonyl) ethane	0.20	0.52	0	0.89	1.14	0
206 2,4,5-Trichloro-3-methyl-6-nitrophenol	0.20	0.54	0	0.94	1.14	0
Control No. 1	0.20	0.56	-	0.85	1.13	-
Control No. 2	0.20	0.45	-	0.80	1.12	-

*averaged fluoromicrophotometric relative intensity readings

APPENDIX C

WATER CHEMISTRY DATA
FIELD TESTS

Chowan River - First Test

NO_3-N
microgram-atoms per liter

Sample	0-day	0+2 days	0+3 days	0+4 days	0+5 days	0+11 days
Ambient River Water	—	0.30	0.25	0.21	0.09	0.23
Control Vessel No. 1	0.80	0.31	0.18	0.25	0.16	0.29
Control Vessel No. 2	0.36	0.35	0.22	0.46	0.29	0.42
Compound No. 23 - 0.8 ppm	0.81	0.26	0.12	0.14	0.44	0.28
Compound No. 23 - 1.6 ppm	0.60	0.66	—	0.85	0.26	0.47
Compound No. 73 - 0.8 ppm	0.28	0.18	0.45	2.04	0.11	0.45
Compound No. 73 - 1.6 ppm	0.16	0.12	0.26	0.27	0.19	0.47

Chowan River - First Test

NH_4-N
microgram-atoms per liter

Sample	0-day	0+2 days	0+3 days	0+4 days	0+5 days	0+11 days
Ambient River Water	—	2.85	2.50	5.52	2.19	3.12
Control Vessel No. 1	2.88	2.98	3.26	10.78	2.98	4.46
Control Vessel No. 2	2.43	4.36	2.95	6.08	3.57	9.58
Compound No. 23 - 0.8 ppm	8.52	5.73	5.80	4.29	4.56	5.15
Compound No. 23 - 1.6 ppm	9.51	11.40	7.31	6.01	3.74	10.1
Compound No. 73 - 0.8 ppm	2.46	3.70	5.97	4.02	2.78	5.15
Compound No. 73 - 1.6 ppm	3.36	2.67	3.36	3.19	3.67	5.60

Chowan River - First Test

NO_2-N
microgram-atoms per liter

Sample	0-day	0+2 days	0+3 days	0+4 days	0+5 days	0+11 days
Ambient River Water		0.07	0.08	0.09	0.07	0.08
Control Vessel No. 1	0.08	0.08	0.07	0.08	0.07	0.08
Control Vessel No. 2	0.08	0.09	0.09	0.09	0.08	0.08
Compound No. 23 - 0.8 ppm	0.32	0.18	0.27	0.09	0.07	0.09
Compound No. 23 - 1.6 ppm	0.44	0.78	0.32	0.10	0.08	0.08
Compound No. 73 - 0.8 ppm	0.08	0.14	0.08	0.08	0.07	0.09
Compound No. 73 - 1.6 ppm	0.07	0.13	0.08	0.08	0.09	0.08

Chowan River - First Test

REACTIVE PO_4
microgram-atoms per liter

Sample	0-day	0+2 days	0+3 days	0+4 days	0+5 days	0+11 days
Ambient River Water	—	0.24	0.26	0.34	0.26	0.40
Control Vessel No. 1	0.68	—	0.16	0.46	0.26	0.54
Control Vessel No. 2	0.22	0.58	0.26	0.48	0.48	0.72
Compound No. 23 - 0.8 ppm	0.58	0.16	0.14	0.35	0.14	0.48
Compound No. 23 - 1.6 ppm	0.64	1.36	0.10	0.66	0.36	1.06
Compound No. 73 - 0.8 ppm	0.84	0.20	0.30	0.38	0.32	0.56
Compound No. 73 - 1.6 ppm	0.16	0.26	0.26	0.34	0.36	0.74

Chowan River - First Test

TOTAL FILTERABLE PO_4
microgram-atoms per liter

Sample	0-day	0+2 days	0+3 days	0+4 days	0+5 days	0+11 days
Ambient River Water	---	0.80	0.66	0.94	0.96	1.24
Control Vessel No. 1	1.80	0.84	1.20	1.24	0.78	1.22
Control Vessel No. 2	0.70	1.46	0.90	1.38	1.10	1.50
Compound No. 23 - 0.8 ppm	1.14	0.74	0.44	1.38	0.90	0.96
Compound No. 23 - 1.6 ppm	1.14	2.84	0.64	1.50	1.06	2.10
Compound No. 73 - 0.8 ppm	1.50	0.94	1.16	1.20	0.84	1.10
Compound No. 73 - 1.6 ppm	1.20	0.86	0.66	0.94	1.06	1.74

Chowan River - First Test

TOTAL UNFILTERABLE PO_4,
microgram-atoms per liter

Sample	0-day	0+2 days	0+3 days	0+4 days	0+5 days	0+11 days
Ambient River Water	—	2.74	2.30	2.60	2.54	3.10
Control Vessel No. 1	2.68	3.14	2.24	2.58	2.72	3.54
Control Vessel No. 2	1.96	3.30	2.16	3.06	2.62	4.00
Compound No. 23 - 0.8 ppm	2.16	2.76	2.30	2.80	2.36	3.40
Compound No. 23 - 1.6 ppm	2.24	4.46	2.36	3.40	2.76	5.10
Compound No. 73 - 0.8 ppm	3.50	2.20	2.52	3.00	2.56	3.54
Compound No. 73 - 1.6 ppm	2.24	2.14	2.02	2.90	3.12	3.56

Chowan River - First Test

TOTAL CHLOROPHYLL
microgram-atoms per liter

Sample	0-day	0+2 days	0+3 days	0+4 days	0+5 days	0+11 days
Ambient River Water	12.43	33.84	22.34	27.00	24.61	17.17
Control Vessel No. 1	11.02	10.01	25.57	22.49	21.70	22.97
Control Vessel No. 2	7.15	21.08	22.34	26.16	19.24	40.08
Compound No. 23 - 0.8 ppm	6.80	8.33	4.00	32.66	28.62	28.78
Compound No. 23 - 1.6 ppm	1.40	4.80	3.40	22.42	26.98	31.03
Compound No. 73 - 0.8 ppm	36.58	7.11	7.01	31.50	16.03	30.83
Compound No. 73 - 1.6 ppm	11.08	3.38	4.44	14.50	17.96	28.63

Chowan River - First Test

Agmenellum AND Oscillatoria
(cells/liter x 10[illegible])

Sample	0-days	0+1 day	0+4 days	0+11 days
Ambient River Water	$\frac{45}{3}$	$\frac{9}{6}$	$\frac{48}{58}$	$\frac{95}{4}$
Controls (Avg.)	$\frac{52}{11}$	$\frac{70}{11}$	$\frac{106}{11}$	$\frac{120}{6}$
Compound No. 23 - 0.8 ppm	$\frac{29}{10}$	$\frac{72}{0}$	$\frac{58}{0}$	--
Compound No. 23 - 1.6 ppm	$\frac{88}{10}$	$\frac{59}{3}$	$\frac{31}{2}$	--
Compound No. 73 - 0.8 ppm	$\frac{45}{8}$	$\frac{53}{1}$	$\frac{13}{5}$	$\frac{90}{4}$
Compound No. 73 - 1.6 ppm	$\frac{36}{10}$	$\frac{29}{0}$	$\frac{94}{4}$	$\frac{13}{7}$

KEY: $\frac{\text{Agmenellum}}{\text{Oscillatoria}}$

Lake Sallie - First Test

TEMPERATURE AND pH VARIATIONS

Sample	0-days	0+2 days	0+4 days	0+6 days
Ambient Lake Water	$\frac{25.5}{8.4}$	$\frac{24.5}{8.9}$	$\frac{23.0}{8.8}$	$\frac{24.0}{8.9}$
Control Vessel No. 1	--	--	--	--
Control Vessel No. 1	$\frac{25.5}{9.0}$	$\frac{24.5}{8.9}$	$\frac{23.0}{8.8}$	$\frac{24.0}{8.9}$
Compound No. 23 - 0.8 ppm	$\frac{25.5}{9.0}$	$\frac{24.0}{8.9}$	$\frac{22.5}{8.8}$	$\frac{24.0}{8.9}$
Compound No. 23 - 1.6 ppm	$\frac{25.5}{9.0}$	$\frac{24.0}{8.9}$	$\frac{22.5}{8.8}$	$\frac{24.0}{8.9}$
Compound No. 73 - 0.8 ppm	$\frac{25.5}{9.0}$	$\frac{24.5}{8.9}$	$\frac{23.0}{8.7}$	$\frac{23.5}{8.8}$
Compound No. 73 - 1.6 ppm	$\frac{25.5}{9.0}$	$\frac{24.0}{8.9}$	$\frac{23.0}{8.8}$	$\frac{24.0}{8.9}$

KEY: $\frac{\text{temp., (°C)}}{\text{pH}}$

Lake Sallie - First Test
AVAILABLE AND TOTAL PO_4 (µg/l)

Sample	0-days	0+2 days	0+4 days	0+6 days
Ambient Lake Water	$\frac{0.105}{0.64}$	$\frac{0.01}{0.43}$	$\frac{0.130}{0.43}$	$\frac{0.095}{0.49}$
Control Vessel No. 1	--	--	--	--
Control Vessel No. 2	$\frac{0.105}{0.47}$	$\frac{0.320}{0.45}$	$\frac{0.055}{0.38}$	$\frac{0.170}{0.43}$
Compound No. 23 - 0.8 ppm	$\frac{0.085}{0.64}$	$\frac{0.020}{0.43}$	$\frac{0.085}{0.45}$	$\frac{0.095}{0.43}$
Compound No. 23 - 1.6 ppm	$\frac{0.105}{0.60}$	$\frac{0.085}{0.30}$	$\frac{0.105}{0.43}$	$\frac{0.215}{0.47}$
Compound No. 73 - 0.8 ppm	$\frac{0.075}{0.47}$	$\frac{0.105}{0.56}$	$\frac{0.130}{0.43}$	$\frac{0.095}{0.43}$
Compound No. 73 - 1.6 ppm	$\frac{0.105}{0.62}$	$\frac{0.115}{0.49}$	$\frac{0.020}{0.43}$	$\frac{0.030}{0.38}$

KEY: $\frac{\text{Avail. } PO_4}{\text{Total } PO_4}$

Lake Sallie - First Test

NO_2-N and NO_3-N (µg/1)

Sample	0-days	0+2 days	0+4 days	0+6 days
Ambient Lake Water	$\frac{0}{0.015}$	$\frac{0}{0.017}$	$\frac{0}{0.014}$	$\frac{0}{0.014}$
Control Vessel No. 1	--	--	--	--
Control Vessel No. 2	$\frac{0}{0.014}$	$\frac{0}{0.014}$	$\frac{0}{0.037}$	$\frac{0.001}{0.012}$
Compound No. 23 - 0.8 ppm	$\frac{0}{0.017}$	$\frac{0}{0.050}$	$\frac{0}{0.14}$	$\frac{0}{0.015}$
Compound No. 23 - 1.6 ppm	$\frac{0}{0.015}$	$\frac{0.001}{0.016}$	$\frac{0}{0.015}$	$\frac{0}{0.014}$
Compound No. 73 - 0.8 ppm	$\frac{0}{0.014}$	$\frac{0}{0.014}$	$\frac{0.002}{0.014}$	$\frac{0}{0.014}$
Compound No. 73 - 1.6 ppm	$\frac{0}{0.014}$	$\frac{0}{0.010}$	$\frac{0}{0.014}$	$\frac{0}{0.014}$

KEY: $\frac{NO_2\text{-}N}{NO_3\text{-}N}$

Lake Sallie - First Test

CO_2 AND HCO_3 (µg/1)

Sample	0-days	0+2 days	0+4 days	0+6 days
Ambient Lake Water	$\frac{20}{155}$	$\frac{52}{132}$	$\frac{44}{144}$	$\frac{56}{132}$
Control Vessel No. 1	--	--	--	--
Control Vessel No. 2	$\frac{52}{124}$	$\frac{48}{134}$	$\frac{44}{146}$	$\frac{56}{134}$
Compound No. 23 - 0.8 ppm	$\frac{52}{124}$	$\frac{48}{134}$	$\frac{44}{146}$	$\frac{56}{134}$
Compound No. 23 - 1.6 ppm	$\frac{52}{129}$	$\frac{52}{134}$	$\frac{40}{150}$	$\frac{52}{132}$
Compound No. 73 - 0.8 ppm	$\frac{48}{129}$	$\frac{52}{134}$	$\frac{40}{150}$	$\frac{56}{132}$
Compound No. 73 - 1.6 ppm	$\frac{44}{132}$	$\frac{52}{138}$	$\frac{44}{146}$	$\frac{56}{132}$

KEY: $\frac{CO_2}{HCO_3}$

Lake Sallie - First Test

NH_3-Nitrogen

Sample	0-days	0+2 days	0+4 days	0+6 days
Ambient Lake Water	0.080	0.065	0.035	0.105
Control Vessel No. 1	---	---	---	---
Control Vessel No. 2	0.050	0.030	0.035	0.060
Compound No. 23 - 0.8 ppm	0.115	0.055	0.035	0.090
Compound No. 23 - 1.6 ppm	0.140	0.050	0.035	1.80
Compound No. 73 - 0.8 ppm	0.015	0.030	0.035	0.080
Compound No. 73 - 1.6 ppm	0.065	0.080	0.065	0.090

Muskrat Lake - Second Minnesota Test

NH_3- NITROGEN

Sample	0-days	0+9 days
Ambient Lake Water	0.065	0.100
Control	0.065	0.065
Compound No. 73 - 3.2 ppm	0.080	0.155
Compound No. 73 - 1.6 ppm	0.050	0.065
Compound No. 73 - 0.8 ppm	0.050	0.115
Compound No. 73 - 0.4 ppm	0.050	0.065

Muskrat Lake - Second Minnesota Test

TEMPERATURE AND pH VARIATIONS

Sample	0-days	0+9 days
Ambient Lake Water	$\frac{24.0}{8.5}$	$\frac{20.0}{8.4}$
Control	$\frac{24.0}{8.8}$	$\frac{20.0}{9.0}$
Compound No. 73 - 3.2 ppm	$\frac{24.0}{8.9}$	$\frac{20.0}{8.9}$
Compound No. 73 - 1.6 ppm	$\frac{24.0}{8.8}$	$\frac{20.0}{9.\text{[illegible]}}$
Compound No. 73 - 0.8 ppm	$\frac{24.0}{8.8}$	$\frac{20.0}{9.1}$
Compound No. 73 - 0.4 ppm	$\frac{24.0}{8.8}$	$\frac{20.0}{9.1}$

KEY: $\frac{\text{temp., (°C)}}{\text{pH}}$

Muskrat Lake - Second Minnesota Test
NO_2-N AND NO_3-N (µg/l)

Sample	0-day	0+9 days
Ambient Lake Water	$\frac{0}{0.14}$	$\frac{0.001}{0.023}$
Control	$\frac{0.001}{0.37}$	$\frac{0.001}{0.018}$
Compound No. 73 - 3.2 ppm	$\frac{0}{0.14}$	$\frac{0.001}{0.020}$
Compound No. 73 - 1.6 ppm	$\frac{0}{0.14}$	$\frac{0}{0.024}$
Compound No. 73 - 0.8 ppm	$\frac{0}{0.19}$	$\frac{0.001}{0.017}$
Compound No. 73 - 0.4 ppm	$\frac{0}{0.14}$	$\frac{0.001}{0.026}$

KEY: $\frac{NO_2\text{-N}}{NO_3\text{-N}}$

Muskrat Lake - Second Minnesota Test
CO_3 AND HCO_3 ALKALINITIES (μg/1)

Sample	0-day	0+9 days
Ambient Lake Water	$\frac{24}{144}$	$\frac{12}{172}$
Control	$\frac{56}{130}$	$\frac{52}{124}$
Compound No. 73 - 3.2 ppm	$\frac{60}{126}$	$\frac{48}{114}$
Compound No. 73 - 1.6 ppm	$\frac{52}{122}$	$\frac{72}{110}$
Compound No. 73 - 0.8 ppm	$\frac{52}{120}$	$\frac{60}{113}$
Compound No. 73 - 0.4 ppm	$\frac{60}{130}$	$\frac{60}{115}$

KEY: $\frac{CO_3}{HCO_3}$

Muskrat Lake - Second Minnesota Test
SOLUBLE AND TOTAL PO_4 (µg/l)

Sample	0-day	0+9 days
Ambient Lake Water	$\frac{2.9}{4.6}$	$\frac{1.4}{2.6}$
Control	$\frac{2.6}{2.8}$	$\frac{0.64}{1.2}$
Compound No. 73 - 3.2 ppm	$\frac{2.1}{5.4}$	$\frac{0.96}{1.9}$
Compound No. 73 - 1.6 ppm	$\frac{2.8}{3.2}$	$\frac{0.79}{1.5}$
Compound No. 73 - 0.8 ppm	$\frac{2.6}{2.9}$	$\frac{0.93}{1.7}$
Compound No. 73 - 0.4 ppm	$\frac{2.3}{3.8}$	$\frac{0.75}{1.6}$

KEY: $\frac{\text{Soluble } PO_4}{\text{Total } PO_4}$

Diamond Lake - First Test

GREEN ALGAE - Staurastrum & Gloeocystis

(cells/ml)

Sample	0-days	0+1 day	0+2 days	0+3 days
Ambient Lake Water	$\frac{11.7}{24.5}$	$\frac{14.9}{37.4}$	$\frac{12.1}{13.0}$	$\frac{8.6}{74.4}$
Control Vessel No. 1	$\frac{11.9}{43.2}$	$\frac{10.6}{36.0}$	$\frac{17.6}{44.5}$	$\frac{18.9}{8.6}$
Compound No. 73 - 0.4 ppm	$\frac{13.5}{60.5}$	$\frac{16.7}{29.5}$	$\frac{14.6}{43.9}$	$\frac{17.5}{49.0}$
Compound No. 73 - 0.8 ppm	$\frac{17.1}{30.2}$	$\frac{16.7}{30.2}$	$\frac{14.6}{74.9}$	$\frac{11.9}{33.5}$
Compound No. 73 - 0.8 ppm*	$\frac{15.5}{41.8}$	$\frac{13.7}{47.5}$	$\frac{17.1}{60.5}$	$\frac{16.0}{79.2}$
Compound No. 73 - 1.6 ppm	$\frac{13.0}{30.2}$	$\frac{14.4}{20.2}$	$\frac{16.2}{23.0}$	$\frac{17.1}{46.1}$
Compound No. 73 - 3.2 ppm	$\frac{11.9}{30.2}$	$\frac{13.6}{27.4}$	$\frac{16.6}{40.3}$	$\frac{18.4}{15.8}$

*Solubilized in methyl alcohol

KEY: $\frac{\text{Staurastrum}}{\text{Gloeocystis}}$

Diamond Lake - First Test

Fragilaria AND Eudorina
(cells/ml)

Sample	0-days	0+1 day	0+2 days	0+3 days
Ambient Lake Water	5.8 2.9	9.4 2.9	1.1 5.8	2.5 7.9
Control Vessel No. 1	0 2.2	4.0 2.9	2.9 2.6	11.5 0
Compound No. 73 - 0.4 ppm	0 0	3.1 5.8	9.7 2.9	4.5 10.1
Compound No. 73 - 0.8 ppm	0 0	4.7 2.9	2.7 0	9.5 15.8
Compound No. 73 - 0.8 ppm*	2.7 2.9	3.1 2.9	0 2.9	3.4 2.9
Compound No. 73 - 1.6 ppm	3.6 4.3	4.7 0	5.0 2.9	6.1 0
Compound No. 73 - 3.2 ppm	2.5 0	5.6 4.3	4.0 2.9	6.8 2.2

*Solubilized in methyl alcohol

KEY: Fragilaria
Eudorina

Diamond Lake - First Test

DISSOLVED OXYGEN AND TOTAL ALKALINITY LEVELS

ppm/(mg/l)

Sample	0-days	0+1 day	0+2 days	0+3 days
Ambient Lake Water	$\frac{8.2}{17.0}$	$\frac{--}{15.0}$	$\frac{--}{17.0}$	$\frac{8.5}{17.0}$
Control Vessel No. 1	$\frac{8.1}{17.0}$	$\frac{8.2}{16.0}$	$\frac{8.6}{17.0}$	$\frac{9.0}{16.5}$
Compound No. 73 - 0.4 ppm	$\frac{8.1}{17.0}$	$\frac{8.2}{17.0}$	$\frac{8.5}{17.0}$	$\frac{8.6}{16.5}$
Compound No. 73 - 0.8 ppm	$\frac{8.2}{17.0}$	$\frac{8.2}{16.5}$	$\frac{8.4}{17.0}$	$\frac{8.6}{16.5}$
Compound No. 73 - 0.8 ppm*	$\frac{8.2}{17.0}$	$\frac{8.3}{16.0}$	$\frac{8.4}{17.5}$	$\frac{8.6}{17.0}$
Compound No. 73 - 1.6 ppm	$\frac{8.2}{17.0}$	$\frac{8.2}{16.5}$	$\frac{8.4}{17.0}$	$\frac{8.6}{17.0}$
Compound No. 73 - 3.2 ppm	$\frac{8.2}{17.0}$	$\frac{8.3}{16.0}$	$\frac{8.4}{16.5}$	$\frac{8.6}{17.0}$

*Solubilized in methyl alcohol

KEY: $\frac{\text{dissolved oxygen}}{\text{total alkalinity}}$

APPENDIX D

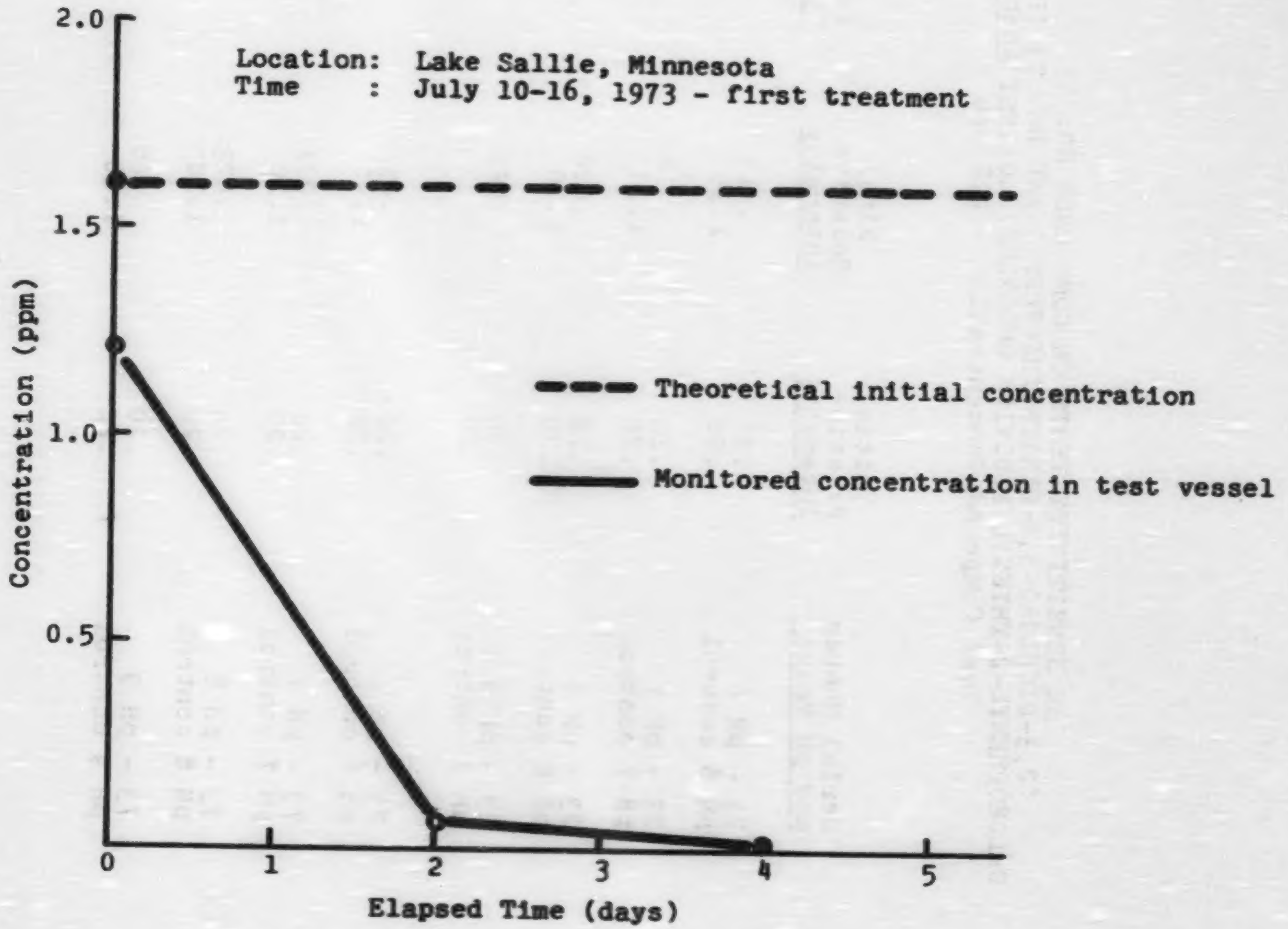
COMPOUND pH-DEPENDENCE AND PERSISTENCE TESTS

pH SENSITIVITY TESTS ON COMPOUNDS NO. 23 (2,3-DICHLORO-3,4-DINITROTHIOPHENE) AND NO. 73 (P-CHLOROPHENYL-2-THIENYL IODONIUM CHLORIDE) AGAINST *Anabaena*

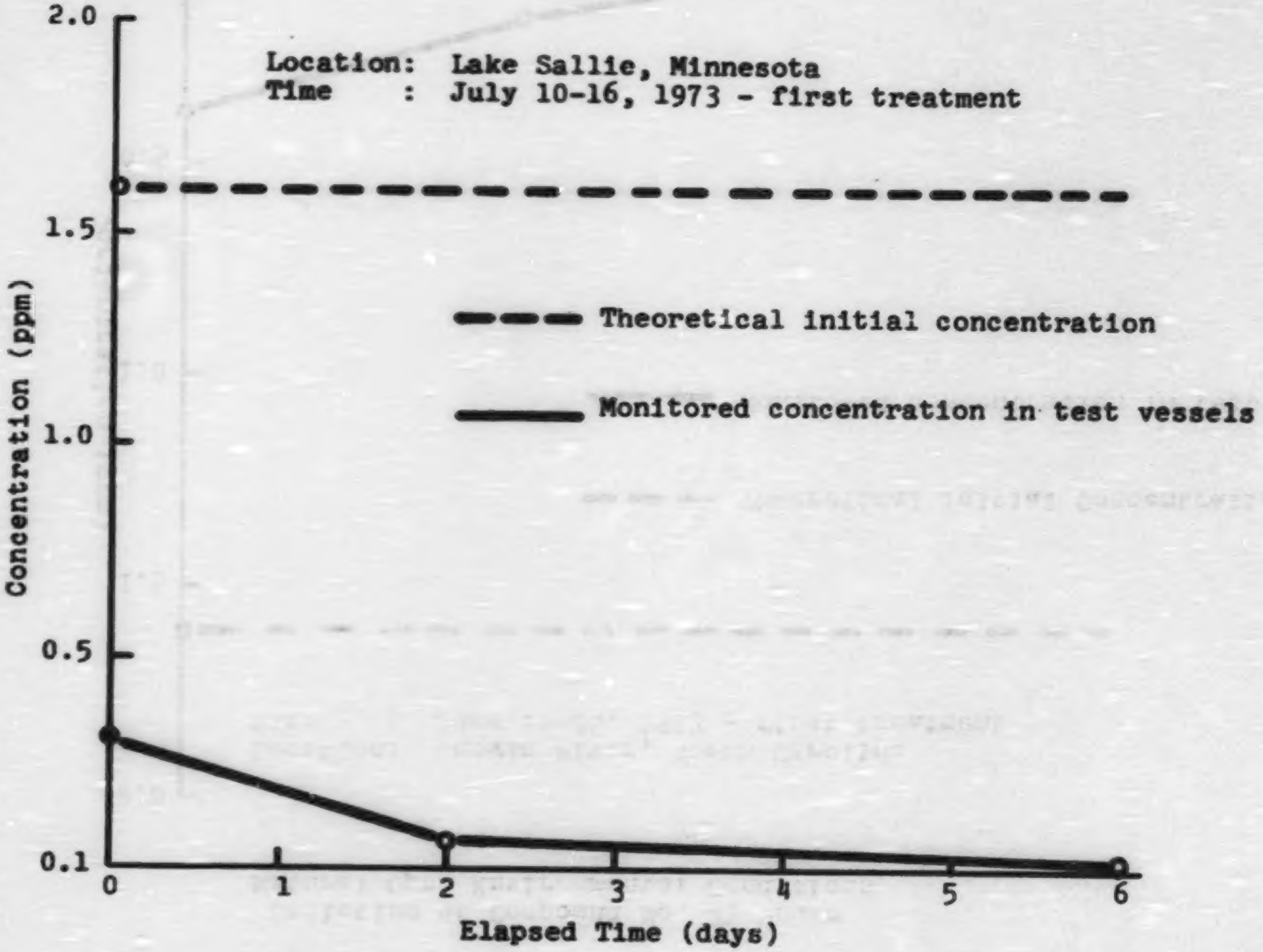
Test Compound Concentration - 0.8 ppm

Serial Number and pH Value	Initial Relative Intensity	Final Relative Intensity	Percent Control
23 - pH 6	.20	.013	100
pH 6 control	.20	1.15	
23 - pH 7	.20	.018	100
pH 7 control	.20	1.18	
23 - pH 8	.20	.020	100
pH 8 control	.20	1.10	
23 - pH 9	.20	.025	100
pH 9 control	.20	1.09	
73 - pH 6	.20	.032	100
pH 6 control	.20	1.40	
73 - pH 7	.20	.021	100
pH 7 control	.20	1.10	
73 - pH 8	.20	.023	100
pH 8 control	.20	1.18	
73 - pH 9	.20	.026	100
pH 9 control	.20	1.10	

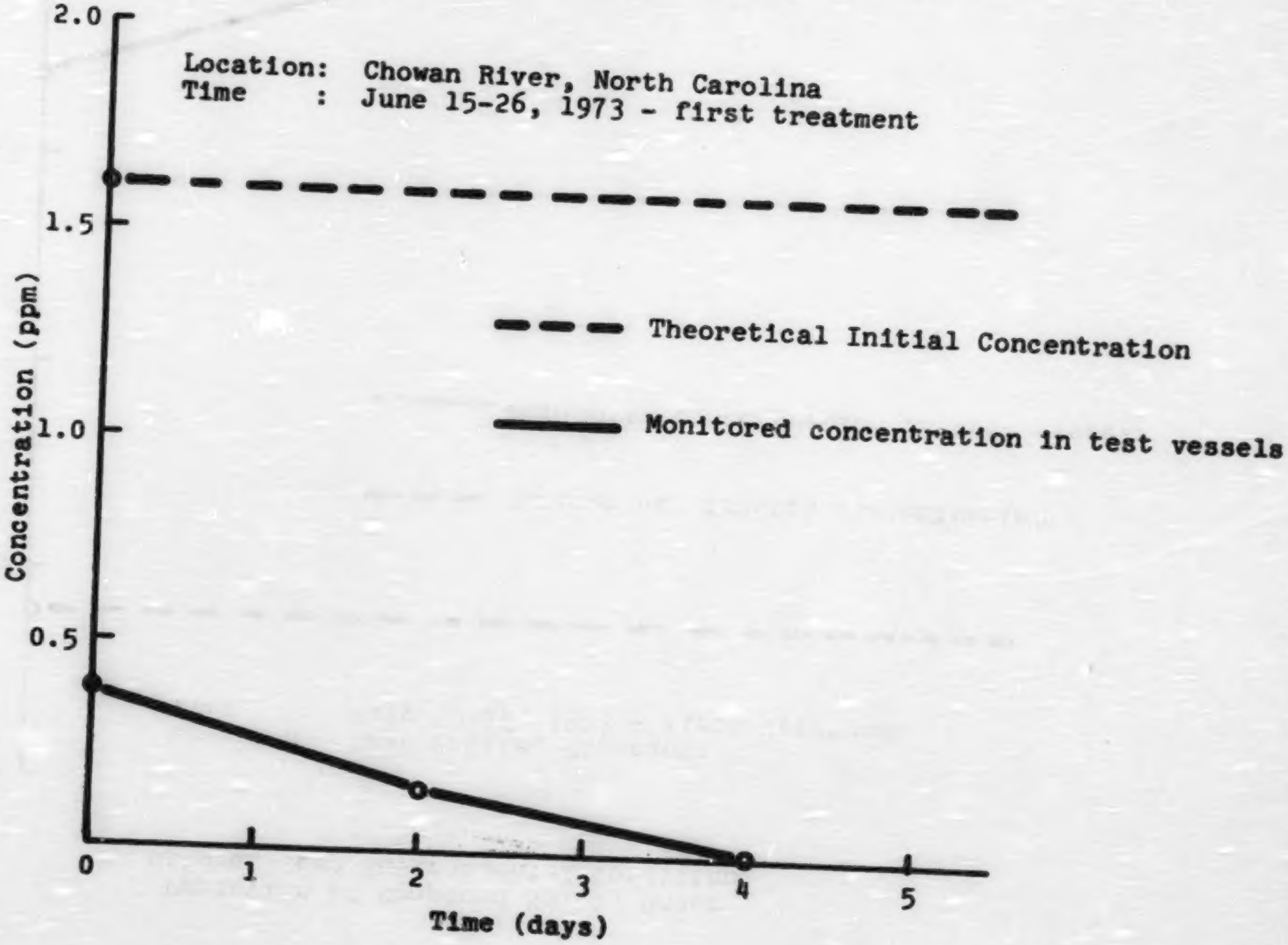
Depletion of Compound No. 73 Under Natural Open Environmental Conditions



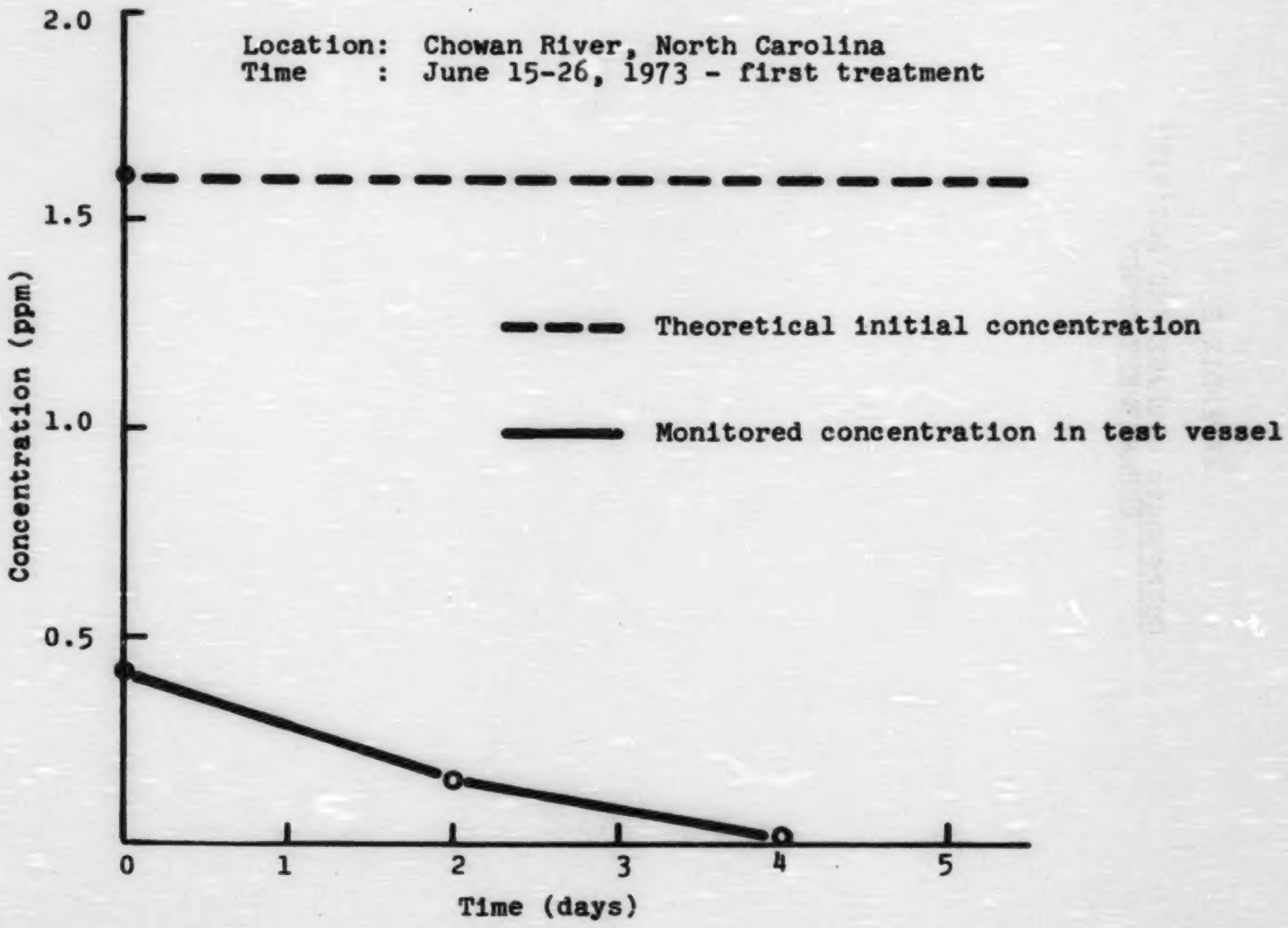
Depletion of Compound No. 23 Under
Natural Open Environmental Conditions



Depletion of Compound No. 23 Under
Natural Open Environmental Conditions



Depletion of Compound No. 73 Under Natural Open Environmental Conditions



APPENDIX E

Ochromonas STORAGE AND ACTIVITY ENHANCEMENT STUDY

Ochromonas STORAGE AND PERSISTENCE STUDY

Parameters:

I. Two species (O. ovalis and O. bastrop)

II. Seven types of media or imbibing substances
  - Gorham's medium
  - Liquid and solid nutrient agar
  - Topsoil
  - River silt
  - Charcoal
  - Polyester fibers
  - Cotton fibers

III. Four states
  - Liquid
  - Dried or partially dried (wet)
  - Sterile
  - Non-sterile

IV. Three temperatures (all in total darkness)
  - Room temperature (23°C)
  - Refrigerator temperature (10°C)
  - Freezer temperature (-4°C)*

V. Storage period - 15 days; subsequent incubation period 3 days

*Samples subjected to below freezing temperatures are not tabulated because in no case did any of the Ochromonas survive.

Ochromonas STORAGE AND PERSISTENCE STUDY
At 0+70 Days

Conditions Which Produced Positive Results	O. ovalis	O. bastrop
Topsoil - refrigerated, sterilized, moist	-	+
Topsoil - room temperature, sterilized, moist	+	-
Activated Charcoal - room temperature, dry	+	-
Activated Charcoal - refrigerated, moist	+	+
Activated Charcoal - room temperature, moist	+	+
Solid Nutrient Agar - room temperature	+	+
Liquid Nutrient Agar - refrigerated	+	-
Liquid Nutrient Agar - room temperature	+	+
Polyester Fibers - room temperature, dry	+	+
Polyester Fibers - room temperature, moist	+	+
Cotton Fibers - room temperature, dry	+	+
Cotton Fibers - refrigerated, moist	-	+
Cotton Fibers - room temperature, moist	-	+

Ochromonas LONGEVITY STUDY

Conditions Which Produced Positive Results at 0+44 Days	O. ovalis	O. bastrop
Activated charcoal - refrigerated - moist	+	-
Activated charcoal - room temperature, moist	+	+
Solid nutrient agar- room temperature	+	+
Liquid nutrient agar - refrigerated	+	+
Polyester fibers - room temperature, dried	-	-
Cotton fibers - room temperature, moist	+	-
Cotton fibers - room temperature, dried	+	+

INFLUENCE OF TEST COMPOUND NO. 119 - N'-(4-((6-bromo-2-pyridinyl)oxy)phenyl)-N,N-dimethylurea AT 0.2 PPM ON THE PHAGOCYTIC ACTIVITY OF FOUR SPECIES OF *Ochromonas*

	CELL COUNTS.								
	0 - days		0 + 1 day		0 + 2 days		0 + 6 days		
Species	(M)x10^{6}	(Och)x10^{6}	(M)x10^{6}	(Och)x10^{6}	(M)x10^{6}	(Och)x10^{6}	(M)x10^{6}	(Och)x10^{6}	Percent Control
O. bastrop+(M)+(T)	0.84	1.0	0.48	2.3	0.0	7.0	0.0	11.0	100
O. danica+(M)+(T)	0.84	1.0	0.61	0.35	0.22	0.80	0.0	10.8	100
O. malhamensis+ (M)+(T)	0.84	1.0	0.74	0.20	0.76	0.80	0.09	5.3	98
O. ovalis+(M)+(T)	0.84	1.0	0.04	4.1	0.0	6.0	0.0	2.0	100
Controls									
O. bastrop+(M)	0.84	1.0	0.63	0.35	0.03	4.5	0.0	12.2	100
O. danica+(M)	0.84	1.0	0.42	0.40	0.53	2.60	0.34	2.0	92
O. malhamensis+(M)	0.84	1.0	0.90	0.50	1.10	0.45	0.75	11.2	82
O. ovalis+(M)	0.84	1.0	0.07	3.5	0.0	8.6	0.0	8.2	100
(M) only Culture No. 1	0.84	—	1.20	—	1.20	—	4.35	—	—
(M) only Culture No. 2	0.84	—	1.16	—	1.06	—	4.25	—	—

Key:

(M) = *Microcystis*
(Och) = *Ochromonas*
(T) = Test chemical

INFLUENCE OF TEST COMPOUND NO. 114 - N, N-diethyl-2-2 (2,4,5-trichlorophenoxy) ethanamine AT 0.2 PPM ON THE PHAGOCYTIC ACTIVITY OF FOUR SPECIES OF *Ochromonas*

	CELL COUNTS								
	0 - days		0 + 1 day		0 + 2 days		0 + 6 days		
Species	(M)x$10^{[illegible]}$	(Och)x$10^{[illegible]}$	(M)x$10^{[illegible]}$	(Och)x$10^{[illegible]}$	(M)x$10^{[illegible]}$	(Och)x$10^{[illegible]}$	(M)x$10^{[illegible]}$	(Och)x$10^{[illegible]}$	Percent Control
O. bastrop+(M)+(T)	0.84	1.0	1.08	0.25	1.08	<.01	1.65	9.5	61
O. danica+(M)+(T)	0.84	1.0	1.07	0.20	0.97	<.01	1.74	15.1	59
O. malhemensis + (M)+(T)	0.84	1.0	0.88	0.20	1.13	<.01	2.58	0.15	40
O. ovalis+(M)+(T)	0.84	1.0	0.91	0.25	1.05	.10	0.08	6.3	98
Controls									
O. bastrop+(M)	0.84	1.0	0.63	0.35	0.03	4.5	0.0	12.2	100
O. danica + (M)	0.84	1.0	0.42	0.40	0.53	2.6	0.34	2.0	92
O. malhemensis+(M)	0.84	1.0	0.90	0.50	1.1	0.45	0.75	11.2	82
O. ovalis + (M)	0.84	1.0	0.07	3.15	0.0	8.6	0.0	8.2	100
Second Order Controls (*Microcystis* only)									
(M) only - Control No. 1	0.84	—	1.20	—	1.20	—	4.35	—	—
(M) only - Control No. 2	0.84	—	1.16	—	1.06	—	4.25	—	—

Key:

(M) = *Microcystis*
(Och) = *Ochromonas*
(T) = Test compound